FATE OF CONTAMINANTS OF EMERGING ENVIRONMENTAL CONCERN (CEECS) DURING DRINKING WATER TREATMENT PROCESSES

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ABSTRACT

Yi Liu: Fate of Contaminants of Emerging Environmental Concern (CEECs) During Drinking Water Treatment Processes
(Under the direction of Howard S. Weinberg)

Many new chemicals, designed to improve quality of life, and their metabolites are released into the aquatic environment through many pathways and become contaminants of emerging environmental concern (CEECs). Six CEECs (atorvastatin, caffeine, fluoxetine, iohexol, tetracycline and tetrabromobisphenol A), selected for their diverse structure and high use, were evaluated at bench scale for removal from water during coagulation and use of powdered activated carbon (PAC), while chlorination and ultraviolet treatment were evaluated for byproduct formation. Although coagulation was found to be ineffective, PAC was effective and is recommended for removing CEECs from drinking water before disinfection. Chlorination and UV byproduct structures are proposed from infusion-based tandem mass spectrometry results. Tetracycline was studied in more detail and chlorination byproducts included chloroform. A kinetic study evaluating chloroform and total organic halogen formation from chlorination of tetracycline at two different pH (7 and 9) revealed elevated levels for both at pH 7.

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LIST OF ABBREVIATIONS

^{1}H NMR	Proton Nuclear Magnetic Resonance
ACS	American Chemical Society
AR	Analytical Reagent
BPA	Bisphenol A
CCL	Contaminant Candidate List
CEEC	Contaminants of Emerging Environmental Concern
CFC	Chlorofluorocarbon
DBPs	Disinfection Byproducts
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DPD	N,N-Diethyl-p-phenylenediamine
DWS	Drinking Water Strategy
DWTP	Drinking Water Treatment Plant
ECD	Electron Capture Detector
EDC	Endocrine Disrupting Compound
ESI	Electro Spray Ionization
FDA	Food and Drug Administration
FOC	Fluorinated Organic Chemical
GAC	Granular Activated Carbon
GC	Gas Chromatography

GC-MS	Gas Chromatography Mass Spectrometry
HAA	Haloacetic Acid
HAN	Haloacetonitrile
HCB	Hexachlorobenzene
HPLC	High Pressure Liquid Chromatography
I.D.	Inner Diameter
ICM	Iodinated X-ray Contrast Agent
K_{ow}	Octanol-Water Partitioning Coefficient
LC	Liquid Chromatography
LC_{50}	Median Lethal Dose
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LGW	Laboratory Grade Water
LP-UV	Low Pressure Ultra Violet
MALDI	Matrix-Assisted Laser Desorption Ionization
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
MP-UV	Medium Pressure Ultra Violet Light
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MtBE	Methyl tert-butylether
Na_2EDTA	Disodium Ethylenendiamine Tetraacetic Acid

NCI	Negative Chemical Ionization
NMR	Nuclear Magnetic Resonance
NOM	Natural Organic Matter
NPDWR	National Primary Drinking Water Regulation
NSDWR	National Secondary Drinking Water Regulation
OWASA	Orange Water and Sewer Authority
PAC	Powdered Activated Carbon
PB	Particle Beam
PBDE	Polybrominated Diphenyl Ether
PCCL	Preliminary Contaminant Candidate List
PFC	Perfluorinated Compounds
PFOA	Perfluorooctanoic Acid
pKa	Acidity Constant
POP	Persistent Organic Pollutants
POU	Point of Use
PPA	Pollution Prevention Act
PPCP	Pharmaceutical and Personal Care Product
PPG	Poly-propylene Glycol
QTOF	Quadrupole Time-of-Flight
SDWA	Safe Drinking Water Act
SOP	Standard Operating Procedure

SPE	Solid Phase Extraction
TBBPA	Tetrabromobisphenol A
THM	Trihalomethane
TOC	Total Organic Carbon
TOX	Total Organic Halogen
TSCA	Toxic Substance Control Act
U.S. EPA	U.S. Environmental Protection Agency
UHP	Ultra High Purity
UNC	University of North Carolina Chapel Hill
USGS	U.S. Geological Survey
USP	United States Pharmacopeial
UV	Ultra Violet Light
UV-Vis	Ultraviolet-Visible Light Spectroscopy
v/v	Volume to Volume
WWTP	Wastewater Treatment Plant

1. INTRODUCTION

Advancement in the science and technology industries has produced thousands of anthropogenic chemicals to enhance the quality of life. These chemicals have contributed to the increase of agriculture productivity, human lifespan and improvement of human and animal health. Along with the increase in production, use, and disposal of these chemicals, much public attention has been directed to their persistence in the environment. Research has shown that there are many pathways for these chemicals to enter the environment but many of which can be prevented. With the improvement in sensitivity and specificity of instrumentation and separation methods, detection of many of these chemicals in the aqueous and terrestrial environments has improved in recent years (Halden 2010). However, the lack of scientific support for the effects of these chemicals and various other reasons has led to a slow development of regulations. Drinking water is one area of concern, since the release of these chemicals into surface waters either from wastewater or runoff has resulted in their detection in drinking water sources. Some studies have shown that some chemicals are attenuated during conventional drinking water treatment (Benotti et al., 2009; Stackelberg et al., 2007; Vieno et al., 2007; Westerhoff et al., 2005) while others suggest their transformation into forms which are not targeted by analytical methods

(Xu et al. 2012; Duirk et al. 2011; Lam et al. 2005; Vanderford et al. 2008; Eriksson et al. 2004; Bedner & MacCrehan 2006a; Bedner & MacCrehan 2006b). If such chemicals, their metabolites or transformation products are present in drinking water, chronic daily consumption might pose human health concerns and draw attention to how best to reduce such exposure. Clearly, the subject of fate and transport of chemicals of anthropogenic origin in natural waters and their effects on human and ecological health is still in its infancy and much remains unknown.

1.1 Contaminants of Emerging Environmental Concern (CEECs)

Chemicals (mostly organic) of anthropogenic origin that are found in natural water are referred to in the literature by several names including 'emerging chemicals of concern', 'micro-constituents', 'contaminants of emerging concern', 'persistent organic pollutants (POPs)', ‘biologically active compounds (BACs)’ 'unregulated contaminants', 'contaminants of concern', 'pharmaceuticals and personal care products (PPCPs)' or 'contaminants of emerging environmental concern' (CEECs). CEECs include PPCPs, natural and synthetic hormones, pesticides, perfluorinated compounds (PFCs), phthalate plasticizers, surfactants, brominated fire retardants, and other persistent and toxic organohalogen compounds (Halden 2010; Bhandari 2008). Within each category, CEECs can be separated into even smaller sub groups.

1.2 Pathway of Entry into Drinking Water

CEECs originate from many different applications such as manufacturing, individual consumption, medical practice, veterinary practice, and agricultural applications and can be released into the environment including drinking water sources through many pathways (Figure 1.1). Human use of different commercial products in daily routines results in solid and liquid waste containing these CEECs which are directed to wastewater treatment plants (WWTP) or landfills. For example, PPCPs are released into WWTPs through excretion from humans, which can be in the forms of a metabolite or the parent compound, or the disposal of it directly into toilets. Wastewater treatment does not guarantee the complete removal of the chemicals nor do landfills maintain an absolute seal over their lifetime (Schwarzbauer et al. 2002) resulting in the release of CEECs into water bodies (Heidler et al. 2006; Kolpin et al. 2002). BACs in the form of drugs administered to humans and animals are incompletely metabolized (Hirsch et al. 1999) but the parent compound and metabolite will be excreted into wastewater (Langford & Thomas 2011). Land applied chemicals such as herbicides and pesticides or even the biosolids from wastewater treatment can runoff into rivers and streams (Ritter et al. 2011). These are some of the pathways that lead to the deposition of CEECs in the environment (Figure 1.1). Table 1.1 lists some of the results of a survey of streams in the US for 95 organic wastewater contaminants including BACs, hormones, flame retardants, etc. all of which are considered CEECs (Kolpin et al. 2002). The frequent

occurrence of some CEECs in this survey raises concerns especially since some of these waters are the drinking water sources of many cities. A median lethal concentration (LC_{50}) is the exposure dose of chemical to a living cell (species) in which half of the cells die and is used to compare the toxicity of chemicals. The lower the LC_{50}, the more toxic a substance is. The indicator species referred to in Table 1.1 differed according to the chemical tested so as to measure the LC_{50} for the most sensitive cells. Some CEECs have LC_{50} values in the μg/L or ng/L range which are the occurrence levels in some surface waters and could pose a threat to human health.

Table 1.1: Occurrence, concentration and toxicity of commonly found CEECs in 139 US streams (Kolpin et al. 2002).

Chemical	**N**	**Frequency (%)**	**Max Concentration (μg/L)**	**LC_{50} for most sensitive indicator species (μg/L)**
Fluoxetine	84	<1	1.2	N/A
Caffeine	85	70.6	5.7	40000
Bisphenol A	85	41.2	12	3800
Ethanol, 2-butoxy-phosphate	85	45.9	6.7	10400
4-nonylphenol	85	50.6	40	130
Triclosan	85	57.6	2.3	4680
Tri(2-chloroethyl)phosphate	85	57.6	0.54	66000
Cholesterol	70	84.3	60	N/A
Coprostanol	70	85.7	150	N/A

LC_{50}: median lethal concentration
N: number of samples
N/A: not available

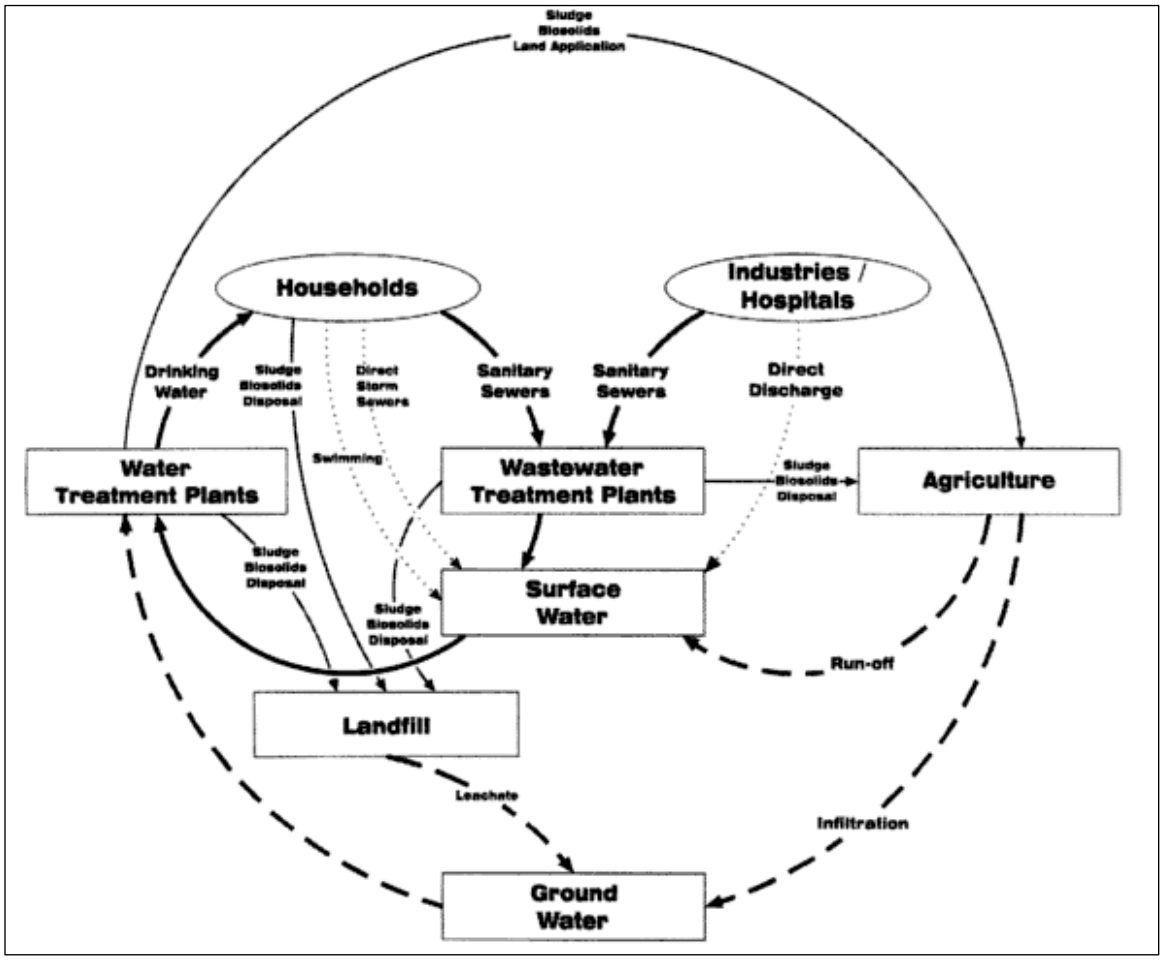


Figure 1.1: Pathways of PPCPs into drinking water and the environment (Bhandari 2008).

Fluorinated organic chemicals (FOCs) are one of the most recently identified emerging groups of CEECs and are found in refrigerants, aerosols, non-stick cookware, lubricants, aviation, and pharmaceuticals. Ritter (2012) stated that "3 out of 10 best selling drugs in 2011 contain fluorine" and "7 out of 35 new drugs approved in 2011 contain fluoride". FOCs have a carbon-fluorine bond, one of the strongest bonds, which is difficult to break down. There are only a few natural degradation pathways for FOCs, so it is inevitable that their occurrence is widespread in the environment. FOCs are characterized as persistent organic pollutants (POPs), which are organic substances that are able to bioaccumulate, transport long distances, pose potential risk to humans and

animals, and are persistent in the environment (Ritter et al. 2011). FOCs have been detected in the air at land and sea all over the Atlantic and Southern Oceans, even in the polar regions (Dreyer et al. 2009), and in many animals (Tomy et al. 2009; Holmström & Berger 2008; Tao et al. 2006). FOCs are known carcinogens, endocrine disrupters, and liver toxicants and cause developmental and reproductive problems (Maras et al. 2006; Martin et al. 2007; Abbott et al. 2007; Lau et al. 2003). FOCs include chlorofluorocarbons (CFCs), which have ozone depletion potential (Molina & Rowland 1974). It is important to understand whether FOCs along with other CEECs detected in drinking water sources can be eliminated by natural or conventional treatment processes or require new treatment processes to remove them.

1.3 Fate of CEECs in the Environment

After being released into the aquatic environment, CEECs can volatilize, adsorb onto sediment or remain in water, but they can also degrade, react with other chemicals, or be consumed by organisms. Figure 1.2 shows the possible pathways for CEECs after their release into the environment. Chemical structure and properties can help predict their fate. Among them, octanol-water distribution coefficient (K_{ow}) and acidity constant(s) (pK_a) are very relevant to a wide range of CEECs.

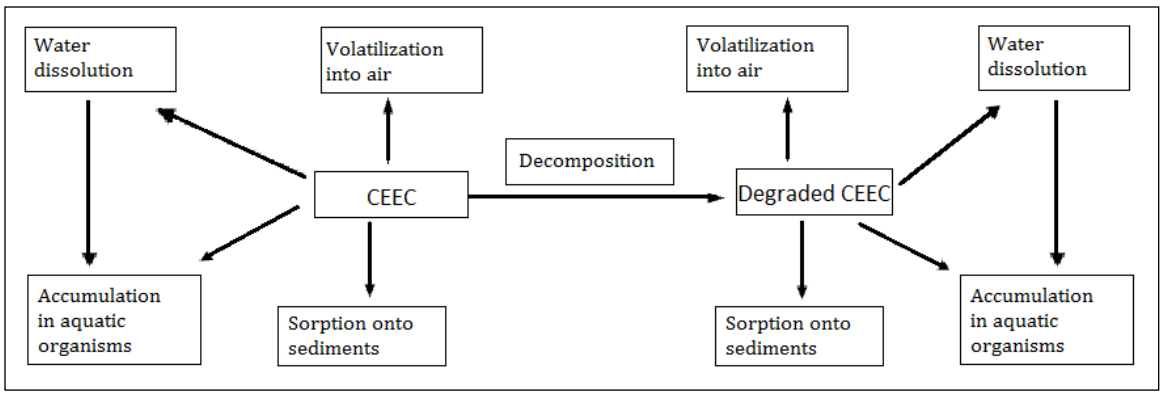


Figure 1.2: Fate of CEECs in the environment.

Log K_{ow} describes the affinity of a compound for the organic (octanol) or aqueous (water) phase when both are present and can assist in predicting whether a chemical will migrate out of water or attach to soil or sediments over which natural water flows. The higher the Log K_{ow}, the more likely a compound is to attach or adsorb onto soil and sediment. Brominated flame retardants have high log K_{ow} values and are, therefore, likely to adsorb onto soil and sediment (De Wit 2002), and be available for biodegradation or uptake by plants. For example, tetrabromobisphenol A (TBBPA) and polybrominated diphenyl ethers (PBDEs) are found at levels 5500 fold higher in the sediment phase compared to the water phase (Labadie et al. 2010). The same reasoning can be applied to CEECs with low log K_{ow} values such as bupropion (anti-depressant, log K_{ow} = 1.54) which is found in water but not in sediment (Schultz et al. 2010).

The pK_a of a compound indicates its speciation in water based on the pH. The compound is protonated when the pH is lower than the pK_a and deprotonated when the pH is higher than the pK_a. In the protonated state, CEECs can be neutral and prefer to be in a more neutral organic phase. When deprotonated and charged, the chemical will

prefer the aqueous phase. Natural waters usually have a pH in the 6-8 range and therefore, compounds such as fluoxetine, iohexol, and TBBPA with a pK_a higher than this will be in the protonated state and exist as a neutral species while compounds such as atorvastatin with a pK_a lower than the aqueous pH will be in the deprotonated state and exist as a charged species. In summary, the two highlighted physiochemical properties suggest that CEECs with high log K_{ow} and pK_a values will be more likely to sorb onto soils and sediment and be removed during wastewater treatment while those with low values are more likely to persist in the aquatic environment (Löffler et al. 2005). Table 1.2 shows the values of log K_{ow} and pK_a for some CEECs.

Table 1.2: Physical and chemical properties of CEECs.

Compound (Trade Name)	**Category**	**Molecular Weight**	**Log K_{ow}[1]**	**pK_a[2]**
Atorvastatin (Lipitor®)	Fluorinated pharmaceutical	558.6	3.76	4.46
Caffeine	Stimulant	194.2	-0.07	6.11
Fluoxetine (Prozac®)	Fluorinated pharmaceutical	309.3	4.57	10.1
Iohexol	X-ray contrast agent	821.1	-3.08	11.4
TBBPA	Flame retardant	543.9	5.20	9.40[3]
Tetracycline	Antibiotic	444.4	-1.39	3.30[4] 7.68[4] 9.69[4]

[1] http://logkow.cisti.nrc.ca/logkow/search.html
[2](Bhandari 2008)
[3](Lezotte & Nixon 2001)
[4](Stephen et al. 1956)

Degradation of CEECs in the environment can occur through mechanisms that include photodegradation and hydrolysis in water, biodegradation in soil and sediments, and reaction with other chemicals in both matrices.

Photodegradation

Photodegradation can be categorized into two types, direct and indirect photolysis (Lam & Mabury 2005). In direct photodegradation, organic compounds absorbing radiation in a sunlit environment become unstable and decompose. Indirect photodegradation involves formation of intermediates such as hydroxyl, carbonate, alkyl peroxy radicals, singlet oxygen, aqueous electrons from nitrate and dissolved organic matter (DOM) that can then react with organic compounds. The type of photodegradation each CEEC undergoes is different depending on its structure and the conditions of exposure. The byproducts formed can also react with each other, making them not well identified, and their effects on human and ecological health are difficult to assess. Eriksson et al. (2004) proposed the major photodegradation byproducts of TBBPA and their degradation pathways, one of which was dehalogenation from the aromatic ring which is a common process in phenols and their derivatives (Richard & Grabner 1999).

Hydrolysis

Hydrolysis is the breakdown of a compound by its reaction with water whose rate depends on the compound structure and conditions, such as pH, temperature, ionic strength and composition (Mabey & Mill 1978). Eight pharmaceuticals including atorvastatin, caffeine, acetaminophen, carbamazepine, levofloxacin, sertraline, sulfamethoxazole, and trimethoprim were determined to not be affected by hydrolysis in a controlled experiment (Lam et al. 2004). However, other CEECs such as oxytetracycline are affected and pH and temperature were found to have an influence on the rate (Xuan et al. 2010).

Biodegradation

Biodegradation is the breakdown of compounds by microorganisms in the environment. Many of the pharmaceuticals, pesticides, and other CEECs were designed to have biological affects and be partially metabolized by their target organisms but in the environment they can also be metabolized by other organisms. In some cases compounds are co-metabolized, in which the compound is modified but not used as a carbon source (Pérez & Barceló 2007). Environmental factors such as temperature and light intensity can also affect chemical biodegradation as shown by Alexy et al. (2004), who assessed 18 antibiotics for biodegradability in a Closed Bottle Test and found that not all were readily biodegradable but eliminated through other mechanisms in the environment. In addition, the authors showed that toxicity was not eliminated for any of

the antibiotics, indicating potential persistence of non-targeted CEEC degradates in surface and possibly drinking water with a lingering biological activity.

1.4 Analytical Methods

The accurate determination of CEEC concentration is important for evaluating their presence in the environment and whether or not they are of concern to human and environmental health. With a wide range of physical and chemical properties, methods developed for CEECs are aimed at clusters such as those with similar polarities but different masses. The mass of a compound and its daughter ions can be determined through tandem mass spectrometry (MS/MS), which is the most commonly used detection technique for CEECs in the environment. Since each chemical has different daughter ions due to the difference in their structures, MS/MS is very useful in identifying the compound of interest. Usually, separation of chemicals in a mixture based on their different physiochemical characteristics proceeds analysis on MS/MS through the use of liquid or gas chromatography (LC or GC). Due to the high boiling points of most CEECs, LC-MS/MS is more widely used.

Due to their low levels in environmental samples, it is essential to concentrate the analytes and clean up the extracts because matrix effects can alter the response of a compound. For water samples, the most commonly used isolation technique is solid

phase extraction (SPE), where a solid phase is first conditioned with solvent, a large volume of pretreated water sample is passed and then the target compound eluted with solvent. The choice of solid phase depends on the analytes of interest. For capturing a broad range of compounds, a hydrophilic lipophilic balanced (HLB) or C-18 solid phase is often used with an eluting solvent of methanol and methyl tert-butylether (MtBE) (Westerhoff et al. 2005; Vanderford & Snyder 2006; Lee et al. 2009). While the stationary phase is selective for certain chemicals, any compound with the same characteristics will also be retained and interfere with the recovery of the target compounds. Once isolated into an organic solvent, the extract can be analyzed by LC- or GC-MS/MS with a high level of specificity. Westerhoff et al. (2005) evaluated 62 different PPCPs and endocrine disrupting compounds (EDCs) during simulated drinking water treatment using SPE paired with LC-MS/MS or GC-MS/MS. As the environmental levels of new CEECs are considered, existing analytical approaches can be adapted for their identification, although full quality controlled methods often lag behind the creation and production of many new anthropogenic chemicals. Since occurrence data depend on method development, there is little information for many CEEC and more study to update methods is required.

1.5 Occurrence in Aquatic Environments

Occurrences of many CEECs in natural water, wastewater sources, and effluents have been reported in many studies (Barclay et al. 2012; Halden 2010; Kaplan 2011; Kormos et al. 2011; Labadie et al. 2010; Langford & Thomas 2009; Lee et al. 2009; Pérez & Barceló 2007; Schultz et al. 2010) (Table 1.3). These pollutants are present often at close to or below detection limits of the existing analytical methods, yet low level chronic exposure effects on human and ecological health are not very well understood. These gaps in our knowledge raise concerns over the fate of CEECs in drinking water treatment and whether they would be present in finished drinking water.

In order to obtain data on occurrence, methods have to be developed and tested for environmental samples. A method for analyzing TBBPA and bisphenol A (BPA) was developed by Zhao et al. (2010) using purified water and then tested with real environmental samples. The environmental samples tested were tap water and wastewater from Jinan, China. The method used bamboo-activated charcoal for SPE extraction and had a recovery of 80.5-119.8% with a limit of detection of 0.01-0.02ng/L. TBBPA and BPA were both detected in the wastewater, but below the detection limit in tap water.

Table 1.3: Occurrence of CEECs in environmental matrices.

Compound Name	Sample Media	Concentration	Reference
Atorvastatin (Lipitor®)	WWTP influent	22-263ng/L	(Lee et al. 2009)
	WWTP effluent	10-122ng/L	
	Drinking water source	0.80-1.4ng/L	(Benotti et al. 2009)
	Drinking water	ND	
Fluoxetine (Prozac®)	River water	1.6-43.2ng/L	(Schultz et al. 2010)
	Sediment	0.39-19.37ng/g	
	Fish brains	0.293-1.648ng/g	
Caffeine	WWTP influent	54-120µg/L	(Stackelberg et al. 2007)
	WWTP effluent	0-50ng/L	
	Drinking water	0.015µg/L	(Yang et al. 2011)
	Streams	0-0.19µg/L	
Iohexol	WWTP influent	3.3-20µg/L	(Kormos et al. 2011)
	WWTP effluent	1.1-1.3µg/L	
	River water	13-69ng/L	
	Drinking water	0-5ng/L	
	Riverbank wells	>3ng/L	
Tetracycline	Stream	0-0.11µg/L	(Kolpin et al. 2002)
	WWTP primary effluent	68-310ng/L	(Yang et al. 2011)
	WWTP effluent	<10ng/L	
Tetrabromobisphenol A	River water	0-64pg/L	(Labadie et al. 2010)
	Sediment	65-28 pg/g	
	Wastewater	0.013-0.03 µg/L	(Zhao et al. 2010)
	Drinking water	>0.01-0.02µg/L	

ND=Not detected

Benotti et al. (2009) evaluated the occurrence of a total of 51 chemicals, including twenty pharmaceuticals, twenty-five EDCs, and six other wastewater contaminants in the source water, finished water and water from distribution systems of 19 DWTPs in the US between 2006 and 2007. These DWTPs received a wide range of source waters

including groundwater, water bodies with or without input from WWTPs and water bodies that allow or do not allow recreational activity. Out of the 51 chemicals, 34 were detected at least once, while 11 were detected more than half of the time. Every DWTP source water sample had at least one compound detected. Source waters that did not have WWTP or recreational use input recorded the lowest number of compounds detected. This survey demonstrated that a well-protected drinking water source had a lower occurrence of CEECs.

Yang et al. (2011) evaluated a wastewater reclamation plant in Gwinnett County, GA. This WWTP train includes a primary clarifier, aeration tanks, secondary clarifier, membrane filtration or granular media filter, granulated activated carbon (GAC) filter, and ozone contactor. Wastewater effluent was collected and analyzed for 19 pharmaceuticals for twelve months. Samples were also collected after unit treatments of primary clarifier (primary effluent), membrane filtration (membrane effluent), and GAC adsorption (GAC effluent). Caffeine was one of the CEECs found at the highest concentrations in the primary effluent (80,000ng/L) and was also found frequently in the final effluent (0-50ng/L). Tetracycline was found in the primary effluent at 160ng/L, but was below the detection limit of 10ng/L after microfiltration. This study showed that wastewater treatment is not always effective in removing all CEECs and some will be discharged into the aquatic environment.

Other than occurrence data, water cycles are evaluated to determine where pollutants accumulate and what forms they transform into. The urban water cycle

(WWTP to surface water to ground water and drinking water) was evaluated for 4 iodinated x-ray contrast media (ICM) and 46 biotransformation products of these ICMs (Kormos et al. 2011). Samples were taken from WWTPs after different unit processes, the discharge river or river wells, and 4 at different points within DWTPs. There was an 80% ICM removal for conventional wastewater treatment but transformation products were detected in wastewater effluent, river water, river wells, and drinking water. The incomplete mass balance analysis of the ICMs and their transformation products suggests that there are many unidentified transformation products unaccounted for by the analytical methods employed.

1.6 Fate of CEECs in Drinking Water Treatment

There is a reduction in concentration of targeted CEECs from source water to finished water in drinking water treatment (Table 1.3), but this decrease is not in accordance with the conservation of mass. Since many studies use methods that are very specific to target compounds, untargeted transformation products and metabolites will not be detected. A reduction in concentration of a chemical after a particular treatment does not elucidate whether the decrease is due to transformation or removal. Studies have shown that some chemicals undergo transformation through photolysis, hydrolysis, biodegradation and chemical reaction (Halden 2010), which make tracking of

chemicals during treatment difficult and often result in incomplete mass balances. Photolysis, hydrolysis and biodegradation were discussed in Section 1.3. Chemical reaction will be discussed later in this chapter. The transformation products are not well understood in either their structure or biological activity. Although some byproducts of a few CEECs such as acetaminophen and iohexol have been identified (Bedner & MacCrehan 2006b; Duirk et al. 2011), many remain unknown. Some of these byproducts have potential toxicity higher than the parent compounds. Therefore, the processes of drinking water treatment need to be investigated not only for the removal of the parent chemicals but following each chemical treatment process, the byproducts need to be identified together with their toxicity.

1.6.1 Chemical Coagulation

Chemical coagulation aids in the removal of particulate contaminants and some dissolved contaminants including suspended solids, colloids, microbes and natural organic matter (NOM). Aluminum sulfate and ferric sulfate, two of the most commonly used chemical coagulants; destabilize particles by neutralizing their charge so that they can agglomerate during flocculation. Coagulant effectiveness is dependent on pH because it affects the formation of the hydrolyzed coagulant which assists with

precipitation. Therefore, it is essential to use the optimal pH range for each coagulant which is 5.5 – 7.7 (aluminum sulfate) and 5 – 8.5 (ferric sulfate) (Crittenden 2005).

Chemical coagulation is not very effective in removing trace-levels of organic pollutants, with the exception of those with high log K_{ow} value (Westerhoff et al. 2005). Such compounds will attach to suspended solids or NOM during coagulation and flocculation and be settled or filtered out. Such is the case with cholesterol (log K_{ow} 8.74) found at a high concentration (7100μg/kg) in dried settled sludge, whereas sulfamethoxazole, acetaminophen, and dehydronifedipine (each with log $K_{ow} < 1.0$) were not detected (Stackelberg et al. 2007). However, log K_{ow} is not the only predictor of CEEC removal which depends also on the pH of the water, concentration of organic matter, properties of the water and the functional groups of the CEEC (Choi et al. 2008). The pH of the water can change the form of CEEC in the water, as discussed in Section 1.3, and impact the effectiveness of coagulation on its removal from water. High organic matter concentrations lead to competition with the CEEC and the coagulant. Choi et al. (2008) found that the removals of tetracycline antibiotics were different for synthetic water and river water, suggesting that the properties of the water are important in determining the removal. Multiple tetracycline antibiotics with similar structures to each other were affected differently by coagulation, suggesting that the different functional groups play a role in their interaction with coagulants.

1.6.2 Powdered Activated Carbon (PAC)

PAC typically used for the removal of taste and odor producing compounds and low concentrations of organic micropollutants, including CEECs, can be used at different points in drinking water treatment before the filtration process. Although it can be easily added to treatment as a slurry or as dry powder, PAC is costly and, therefore, only used when needed. PAC acts through adsorption of organic pollutants via Van der Waals forces. Addition of PAC during coagulation enhances their removal during sedimentation, demonstrated by the increase in removal of tetracycline from ~75% to 100% during coagulation when PAC was added prior to filtration (Knappe et al. 2012). This increases the quality of the water before filtration, thereby preserving the filter efficacy and lifetime.

PAC has been shown to greatly remove many CEECs due to its high absorptivity. The type of material used in the production of PAC impacts the removal of pollutants. The most commonly used are coal based, but others are wood and coconut based. Different PACs have different removal efficiencies for CEECs; however, the difference in removal between the types of PACs is not as significant compared to the difference in removal between different particle sizes of PACs. The smaller the particle size the larger the surface area for absorption, which leads to a higher removal of contaminants and a shorter time needed for equilibration as demonstrated on tests with sulfamethoxazole and trimethoprim (Knappe et al. 2012). One of the powdered carbons was pulverized into

superfine particles resulting in a much higher and more rapid removal of sulfamethoxazole (> 60%) and trimethoprim (100%) than when used in its commercially available form.

1.6.3 Disinfection

Disinfection is often one of the last processes before the treated drinking water is distributed to consumers and is designed to kill bacteria and inactivate pathogens. The disinfectant can be added either before filtration to prevent microbial growth in the filter, or in the clearwell, or both. There are three general types of disinfection: heat treatment (boiling water), radiation treatment (UV radiation) and chemical treatment (chlorination, chloramination, ozone, etc.) (Drinan & Spellman 2013). Heat treatment is used at the point of use when the distribution system is compromised and is, therefore, not commonly used in water treatment plants. Effective disinfectants are required to act in a reasonable time, be nontoxic, not be affected by temperature or pH changes and have a lasting residual during distribution to prevent microbial growth in the pipes. The disinfectants commonly used can only meet some of these characteristics and are, therefore, sometimes used in combination to provide optimal results. Chlorine is the most commonly used disinfectant, but due to its reactions with NOM in the water and creation of harmful disinfection byproducts (DBPs) including trihalomethanes (THMs),

haloacetic acids (HAAs), and haloacetonitriles (HANs) among others (Duirk et al. 2011; Xu et al. 2012; Wang et al. 2011), chloramination has been used as a replacement because lower concentrations are produced (Hua & Reckhow 2007) and the water can be in compliance with the current DBP regulations.

Chlorination

Free chlorine is usually produced from liquid sodium hypochlorite (NaOCl) solution, which reacts with water to produce the oxidants OCl^- and HOCl (Equation 1 and 2), with HOCl being the stronger disinfectant species. A certain contact time is needed for effective inactivation or death of pathogens partially due to the chlorine demand of the water, which needs to be met before a residual can occur. Chlorine reacts with ammonia to form combined chlorine, which includes mono-, di-, and trichloramine. This reaction has been studied extensively and includes the breakpoint phenomenon in which for every one mole of ammonia as ammonium-N, 1.5 moles of free chlorine as Cl_2 is needed after which any additional chlorine added is available for disinfection (White 1999).

$$NaOCl + H_2O \rightleftharpoons HOCl + NaOH \qquad \text{(Equation 1)}$$

$$HOCl + H_2O \rightleftharpoons OCl^- + H_3O^+ \quad (pKa = 7.5) \qquad \text{(Equation 2)}$$

Chlorination has been shown to be effective in the "removal" of many CEECs or at least the parent CEEC molecule is often not found in the chlorine-treated water (Westerhoff et al. 2005; Stackelberg et al. 2007; Kaplan 2011; Knappe et al. 2012). CEECs can have some of the functional moieties of NOM and are, therefore, likely to

produce DBPs upon reaction with chlorine (Pinkston & Sedlak 2004; Bedner & MacCrehan 2006a; Wang et al. 2011; Xu et al. 2012). Chlorine acts as an electrophile and attacks areas on the molecules that are rich in electron density, which include aromatic rings with extended π conjugation and found in many CEECs that can act as nucleophiles and react with chlorine through electrophilic aromatic substitution. The substituents on the benzene ring affect its reactivity through activation or deactivation of the ring for electrophilic attack, as well as the position of the electrophile attack on the substituted benzene ring (Table 1.4). Steric hindrance does not usually affect electrophilic attacks unless the electrophile is large, in which case attack at the para position would be favored over the ortho position, but in the case of chlorine this is not an issue. Steric hindrance of the substituents on the benzene ring does, however, affect reactivity. For example caffeine, which has many substituents on the aromatic ring, will not be as reactive as atorvastatin which has few substituents on the aromatic rings. Figure 1.3 shows a typical electrophilic aromatic substitution with halogen.

Table 1.4: Electrophilic aromatic substitution- effect of substituents (Brown et al. 2005).

Substituent	**Reactivity**	**Directing Orientation**
-NH_2, -NHR, -NR_2, -OH, -OR	Strongly activating	Ortho, Para
-NHCOR, -NHCOAr, -OCOR, -OCOAr	Moderately activating	Ortho, Para
-R, -C_6H_6	Weakly activating	Ortho, Para
-F, -Cl, -Br, -I	Weakly deactivating	Ortho, Para
-COH, -COR, -COOH, -COOR, -$CONH_2$, -SOOOH, -CN	Moderately deactivating	Meta
-NO_2, -NH_3, -CF_3, -CCl_3	Strongly deactivating	Meta

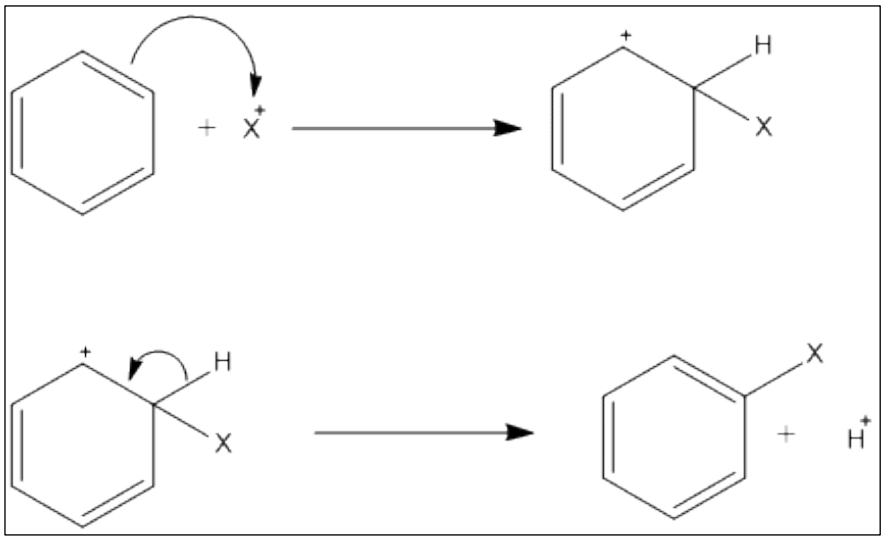


Figure 1.3: Generic electrophilic aromatic substitution with halogen (X) as the electrophile.

Various chlorination reactions of organic compounds have been studied including phenols, dihydroxybenzenes, and amine-containing CEECs (Rebenne et al. 1996; Bedner & MacCrehan 2006a; Gallard & Von Gunten 2002). Chlorination is very reactive with phenols since the –OH group is an activating substituent. Chlorophenols are produced causing concerns for water utilities because of the taste and odor these compounds have (Burttachell et al. 1959). The end product of the reaction is chloroform, which is a THM, but the exact pathway to its formation has not yet been completely elucidated (Gallard & Von Gunten 2002). Chlorination of dihydroxybenzene, which also has –OH substituents, produces chlororesorcinol and dichlororescorcinol (Rebenne et al. 1996). The position at which the chlorine substitution occurs depends on the combined resonance and inductive effects of the ring susbtitutents. Amine-containing CEECs react with chlorine to form a product in which chlorine is added onto the amine group. Fluoxetine, an FOC, reacted with chlorine and produced N-chlorofluoxetine with no aromatic substituted

product identified (Bedner & MacCrehan 2006a). The kinetics and reactivity of these reactions are pH dependent due to the speciation of free chlorine as HOCl and OCl^- defined by the pK_a value and for some CEECs their multiple speciations (e.g. tetracycline has 3 pK_a values).

Chloramination

Chloramines, or combined chlorine, are formed from reaction of chlorine with ammonia. Free chlorine reacts with ammonia to form mono-, di-, and trichloroamine (Equations 3, 4, 5). Monochloramine is formed first followed by dichloramine and then trichloramine as shown in Equation 3, 4, and 5. However, because di- and trichloramine have a strong odor, their formation is controlled and only monochloramine is desirable as a disinfectant. From breakpoint chlorination chemistry, to limit the formation of chloramines to only monochloramine the molar ratio of chlorine to ammonia (as N) needs to be 1:1 and the pH of the water needs to be higher than 9. Compared to free chlorine, monochloramine produces lower levels of regulated DBPs, but because it is a relatively new disinfectant much less is understood about its interaction with NOM or other organics in water, and it may produce more harmful DBPs that are as yet unidentified (Hua & Reckhow 2007).

$$HOCl + NH_3 \rightleftharpoons NH_2Cl + H_2O \quad \text{(Equation 3)}$$

$$NH_2Cl + NH_3 \rightleftharpoons NHCl_2 + H_2O \quad \text{(Equation 4)}$$

$$NHCl_2 + NH_3 \rightleftharpoons NCl_3 + H_2O \quad \text{(Equation 5)}$$

Ultraviolet (UV) Irradiation

UV irradiation acts through the physical process of damaging DNA so that microbes are inactivated (present but not able to cause disease) and unable to reproduce (Crittenden 2005). It cannot be used alone because as a physical process there is no residual from treatment and, therefore, no guarantee that microbes will not subsequently regrow or otherwise enter the distribution lines. Usually a chemical disinfectant is used after UV treatment to solve this problem. There are two types of UV lamps used in disinfection; low-pressure and medium pressure UV. Low pressure UV (LP-UV) outputs light at 254nm while medium pressure UV (MP-UV) outputs at a range of wavelengths between 200 and 400 nm (Lyon 2012). UV can break down contaminants through photolysis and the production of hydroxyl radicals (•OH) that are very reactive, similar to photodegradation as explained in Section 1.3. This can render an otherwise inert chemical into a reactive species that can react with the subsequently added chemical disinfectant to form DBPs (Lyon 2012). This exposes parts of the molecule that usually do not react with chlorine to do so and form more, and perhaps different, DBPs than would otherwise be produced by chlorine alone.

1.7 Identification of By-products

The identification of DBPs is very important to accurately assess the risk of their potentially harmful environmental and health effects. It has been shown that DOM in the water is a precursor to halogenated DBPs such as THMs, HAAs, HANs and many others formed during disinfection with chlorine (Pressman et al. 2010), some of which have been shown to be cytotoxic and/or genotoxic (Richardson et al. 2008; Plewa et al. 2002). CEECs have similar structural moieties to those found in DOM; therefore, they are likely to react with the disinfectant to form chemical byproducts that may harmful to humans. UV disinfection also has been shown to produce many byproducts, which are further chlorinated to produce halogenated DBPs (Lyon 2012). Laboratory studies have been simulated for drinking water and wastewater treatment to determine if CEECs are transformed or eliminated during chlorination and UV treatment (Vanderford et al. 2008; Gould & Richards 1984; Glassmeyer & Shoemaker 2005; Bedner & MacCrehan 2006a; Duirk et al. 2011; Wang et al. 2011; Kaplan 2011; Cermola et al. 2006; Lam et al. 2005; Pereira et al. 2007; Eriksson et al. 2004; Gomez-Pacheco et al. 2012).

The analytical instruments that have been used for byproduct identification include ultraviolet-visible (UV-Vis) light spectroscopy, MS/MS, nuclear magnetic resonance (NMR), and total organic halogen analysis (TOX). UV-Vis can provide a broad scan for byproducts containing chromophores and NMR can help with some structural detail, but MS/MS is the best means of product identification if the product can

be successfully ionized and fragmented into "fingerprint" daughter ions. Structures of the byproducts can be hypothesized from the information obtained. Commercially available standards can then be used to compare to potential byproducts for confirmation purposes. TOX is also a very general method for determining the total amount of halogenated organic species in a product mixture and can be used to indicate whether all halogenated organic byproducts identified individually by GC and LC methods are accounted for. High pressure liquid chromatography (HPLC) or GC is sometimes paired with MS/MS or UV to isolate the byproduct for better sensitivity in detection. However, the limitation with these separation techniques is that some byproducts may not elute off the chromatographic column. A summary of some of the literature on CEEC byproducts from chlorination and UV treatment is shown in Table 1.5 and Table 1.6, respectively.

Table 1.5: Transformation products of CEECs from reaction with free chlorine and their identified toxicity activity.

Compound	Reference	Proposed Byproduct	Analytical Instrumentation	Impact on Biochemical Activity
Atorvastatin	(Vanderford et al. 2008)	Three byproducts identified	QTOF-MS	N/A
Caffeine	(Glassmeyer & Shoemaker 2005)	No change observed	LC-PB-MS	N/A
	(Gould & Richards 1984)	Eight byproducts observed, six were identified	GC-MS, TLC, UV	N/A
Fluoxetine	(Bedner & MacCrehan 2006a)	N-Chlorofluoxetine formed	LC-UV-MS, LC-UV-EC	N/A
Iohexol	(Duirk et al. 2011)	Iodo-THM and Iodo-Acids detected	GC-ECD, GC-NCI-MS	Genotoxic and cytotoxic
Tetracycline	(Wang et al. 2011)	Chlorinated and hydroxylated products without substantial ring breakage	LC-(+)-ESI-MS	activity unknown
	(Kaplan 2011)	Byproducts observed but not identified	LC-MS/MS	N/A

QTOF=Quadrupole time-of-flight
PB=Particle beam
NCI=Negative chemical ionization

Table 1.6: Transformation products of CEECs from reaction with UV treatment and their identified toxicity activity.

Compound	Reference	Proposed Byproduct	Analytical Instrumentation	Impact on Biochemical Activity
Atorvastatin	(Cermola et al. 2006)	Five byproducts identified	HPLC-UV, ^{1}H NMR, MALDI-MS	N/A
Fluoxetine	(Lam et al. 2005)	two byproducts from direct photolysis and two byproducts from indirect photolysis	HPLC-UV, LC-MS/MS	N/A
Iohexol	(Pereira et al. 2007)	No product identified	HPLC-UV, LC-MS/MS	N/A
TBBPA	(Eriksson et al. 2004)	Nine byproducts identified	^{1}H NMR, HPLC-UV, GC-MS	N/A
Tetracycline	(Gomez-Pacheco et al. 2012)	No product identified	Standardized biotest	Byproducts generated at the beginning had higher toxicity, then decreased with longer treatment time, and eventually have a lower toxicity

^{1}H NMR=Proton NMR

MALDI=Matrix-assisted laser desorption ionization

1.8 Policy and Regulation

The Safe Drinking Water Act (SDWA) was created in 1974 to protect public health by regulating the public drinking water supply, and was amended in 1986 and 1996 to include protection of drinking water sources. The U.S. Environmental Protection Agency (U.S. EPA) sets the regulatory limits for contaminants in drinking water under the SDWA and the National Primary Drinking Water Regulations (NPDWRs) lists the limits for more than 90 contaminants in public drinking water (U.S. EPA 2012a). The contaminants include microorganisms, DBPs, disinfectants, inorganic chemicals, organic chemicals, and radionuclides. The limits enforced are called Maximum Contaminant Levels (MCLs) and the recommended limits are called Maximum Contaminant Level Goals (MCLGs). MCLG is the level at which no adverse health effects would be observed while the MCL is the highest level of the contaminant that is allowed. The National Secondary Drinking Water Regulations (NSDWRs), also under the SDWA, list contaminants that may cause cosmetic or aesthetic effects and are recommended for regulations by individual states.

The SDWA also includes a process that identifies unregulated contaminants that may require regulation in the future and periodically publishes a list called the Contaminant Candidate List (CCL) (U.S. EPA 2012b). The CCL helps prioritize the contaminants for scientific research and policy making. CCL 3 is the most current and includes 104 chemicals and 12 microbial contaminants. The contaminants are identified

through a three step process by first identifying the universe of potential drinking water contaminants, and then applying screening criteria to generate a Preliminary CCL (PCCL), which is then further narrowed down to the CCL by detailed evaluation of occurrence and health effects through expert judgment.

In March 2010, the Drinking Water Strategy (DWS) was announced to enhance public health protection from contaminants in drinking water (U.S. EPA 2012c). The objective of the program is to "streamline decision-making, expand protection under existing laws and promote cost-effective new technologies to meet the needs". Four goals were identified: 1) address contaminants as groups, 2) encourage development of new drinking water technologies, 3) use multiple authority statutes to protect drinking water, and 4) develop shared access to monitoring data with states. The U.S. EPA also includes regulations such as the Toxic Substances Control Act (TSCA) passed in 1976 and the Pollution Prevention Act (PPA) enacted in 1990, which control the production and release into the environment of both new and existing chemicals (U.S. EPA 2013). Other than U.S. EPA, the Food and Drug Administration (FDA) regulates chemicals in consumer goods and the U.S. Geological Survey (USGS) monitors chemicals in the environment.

1.9 Research Question

The lack of regulation for most CEECs in drinking water and in the environment is a major concern for the public. In order to determine which CEECs to regulate, there is first a need to identify if the CEEC occurs in the environment and drinking water, then determine if existing treatment processes eliminate the CEEC or creates byproducts, and, finally, determine what risk their presence in the environment (and for the purpose of this study specifically in drinking water) might have on ecological and human health. In this study, the second of the above steps was investigated for drinking water treatment. The occurrence of many CEECs has already been determined but their fate in the drinking water treatment process is not very clear, especially with regards to the formation of byproducts from disinfection treatments. The chemicals that were evaluated in this study include three prescription drugs, a stimulant, an X-ray contrast agent, and a brominated flame retardant, each of which have been consistently found in wastewater effluents, which in turn can become a downstream community's drinking water source. Figure 1.4 shows the structures of the CEECs that were selected for this study.

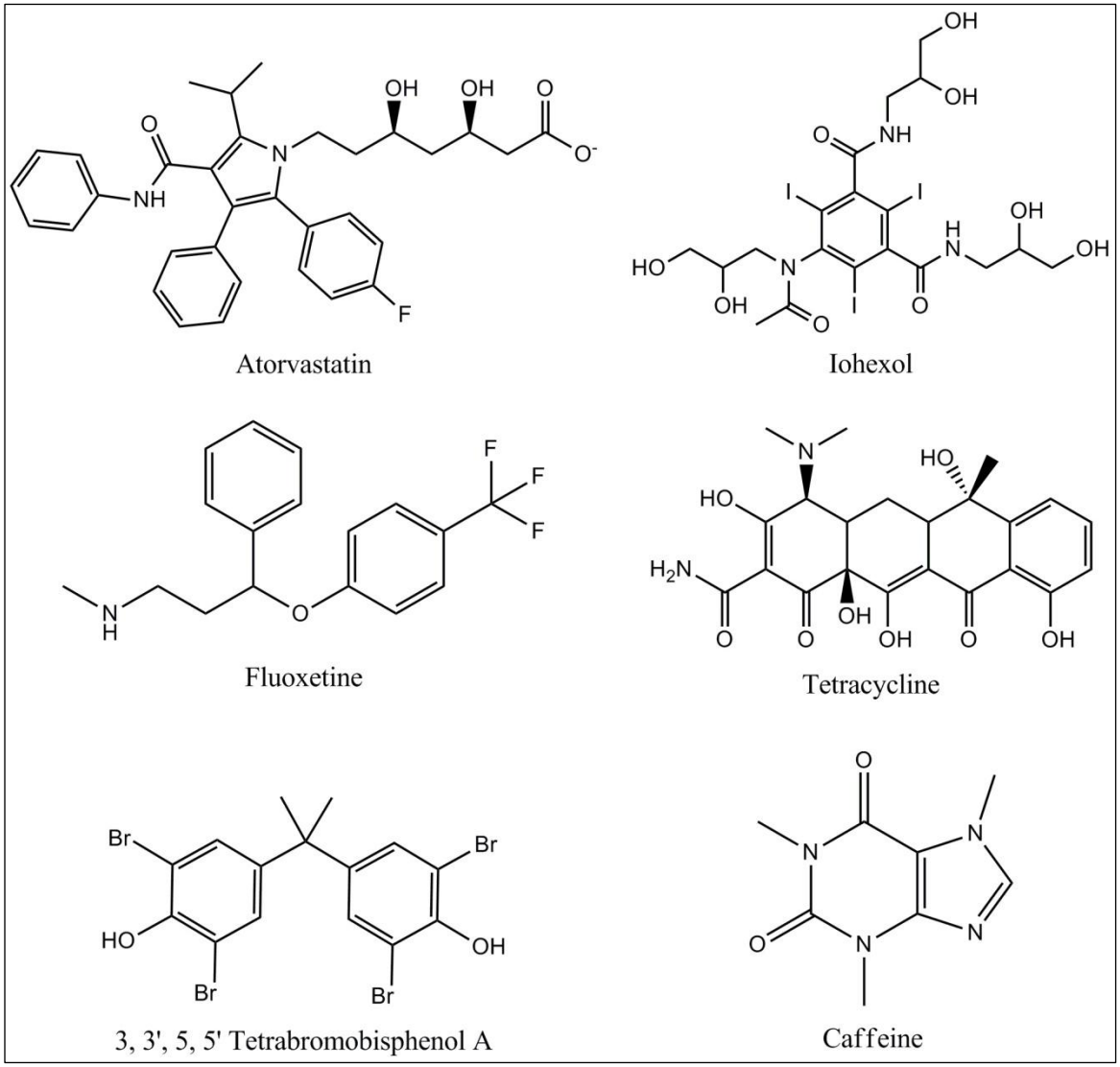


Figure 1.4: Structures of CEECs selected for this study.

1.10 Research Objectives

The objectives of this study were to:

1. determine the effectiveness of removal of a set of CEECs through coagulation and PAC treatment,

2. identify byproducts of disinfection from chlorination and UV treatment of the target CEECs, and
3. study in more detail the effects of chlorination on tetracycline through by-product identification and kinetics.

2. MATERIALS AND METHODS

2.1 Materials

CEEC Standards

Atorvastatin calcium salt trihydrate (≥98%), fluoxetine hydroxide, tetracycline (≥98%), and 97% 3,3',5,5'-tetrabromobisphenol A (TBBPA) were purchased from Sigma-Aldrich (St. Louis, MO). Anhydrous caffeine (≥99%) was purchased from Fluka Analyticals (Buchs, Switzerland). Caffeine-d3 (≥99.8 atom % D) was purchased from C/D/N Isotopes (Pointe-Claire, QC, Canada). Iohexol (388.3mg iohexol/mL) was obtained from UNC hospitals, where it was sourced from Amersham Health (Princeton, NJ). Simeton (100μg/mL) in methanol was purchased from AccuStandard (New Haven, CT). High performance liquid chromatography (HPLC) grade methanol was purchased from Fisher Scientific (Pittsburgh, PA).

Water Used

Laboratory grade water (LGW) was prepared at the University of North Carolina Chapel Hill (UNC) laboratory using a point of use (POU) Dracor Water Systems

(Durham, NC) water purification system. For the POU system, tap water was pre-filtered to 1μm, removed of residual disinfectants, total organic carbon (TOC) was reduced to less than 0.2mg C/L with an activated carbon resin, and ions removed to less than 18MΩ with a mixed bed ion-exchange resin. Surface water samples from Cane Creek Reservoir (Orange County, NC), one of the water sources for Chapel Hill and Carrboro, NC, were collected from Orange Water and Sewage Authority (OWASA) drinking water treatment plant (Carrboro, NC).

Glassware Preparation

All non-volumetric glassware was completely emerged in detergent for 24 hours, washed with LGW, soaked in 10% nitric acid bath for 24 hours, rinsed with LGW, and dried in an oven at 180^{o}C. For volumetric glassware, the same treatment was used but without oven drying. Instead, the glassware was rinsed with certified American Chemical Society (ACS) grade methanol (Fisher, Fair Lawn, NJ) and air dried covered under kimwipes (Kimberly-Clark, Roswell, GA).

Coagulation

Coagulant aluminum sulfate *n*-hydrate (*n*≅aproximate12-14), technical grade, was purchased from Fisher Scientific (Pittsburgh, PA) and ferric sulfate penta-hydrate was purchased from MP Biomedical (Solon, OH). 0.1N sodium hydroxide solution was made from sodium hydroxide pellets purchased from Macron Chemicals (Phillipsburg,

NJ). ACS plus grade concentrated sulfuric acid purchased from Fisher Scientific (Pittsburgh, PA) was used to prepare 0.2N sulfuric acid.

Powdered Activated Carbon (PAC)

Coal-based Calgon WPH 1000 (Pittsburgh, PA) PAC was obtained from OWASA. The particle diameter of the PAC was <0.150mm (99%) and the pore size was 17.2-17.5Å. The 0.7μm glass microfibre filters (934-AH^{TM}) used for filtering samples were purchased from Whatman International Ltd. (Buckinghamshire, England).

Chlorination and Chloramination

Sodium hypochlorite (NaOCl) stock solution as 5.65-6% Cl_2 in water was purchased from Fisher Scientific (Waltham, MA) and the concentration measured monthly using the Iodometric Titration 1 Procedure from Standard Method 408 A (American Public Health Association. 1985). Ammonium chloride, ACS grade, was purchased from Mallinckrodt (St. Louis, MO). Sodium hydroxide, 50% w/w certified was purchased from Fisher Scientific (Pittsburgh, PA). ColorpHast non bleeding pH indicator strips (pH 5-10) were purchased from EMD (Gibbstown, NJ). Chlorine and chloramine residuals were measured using a HACH test kit pocket colorimeter and HACH permachem N,N-Diethyl-p-phenylenediamine (DPD) free/total chlorine reagents (Loveland, CO). Excess chlorine and chloramines were quenched with analytical

reagent (AR) grade anhydrous sodium sulfite (98.9%) purchased from Mallinckrodt (Phillipsburg, NJ) or L-ascorbic acid purchased from Sigma-Aldrich (St. Louis, MO).

Ultraviolet (UV)

Uridine used for actinometry measurements during UV treatment, was purchased from Sigma-Aldrich (St. Louis, MO), while disodium phosphate heptahydrate and sodium phosphate dibasic hepta-hydrate for the phosphate buffer were purchased from Fisher Scientific (Pittsburgh, PA).

Total Organic Halogen (TOX) Analysis

Glacial acetic acid (99.8% certified ACS grade), ACS plus grade concentrated sulfuric acid, potassium nitrate (certified ACS grade), and purified silver acetate used for TOX analysis were all purchased from Fisher Scientific (Pittsburgh, PA). Sodium chloride (~80%) for cell performance checks was purchased from Fluka Analytical (Buchs, Switzerland). The method performance check standard, 2,4,6-trichlorophenol (98%), was purchased from Sigma-Aldrich (St. Louis, MO). ColorpHast non bleeding pH indicator strips (pH 0-2.5) were purchased from EMD (Gisbbston, NJ). AR grade anhydrous sodium sulfite (98.9%) purchased from Mallinckrodt (Phillipsburg, NJ) was used for quenching chlorine residual. Glass-packed activated carbon columns (2mm I.D.) were purchased from CPI international (Santa Rosa, CA). United States

Pharmacopeial (USP) approved oxygen and ultra high purity (UHP) helium were purchased as compressed gases from National Welders Supply, Inc. (Morrisville, NC).

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) Analysis

HPLC grade methanol, OmniSolve methyl-t-butyl ether (MtBE), HPLC grade acetonitrile, and glacial acetic acid (99.8% certified ACS grade) were purchased from Fisher Scientific (Pittsburgh, PA). Formic acid (99.9%) was purchased from Acros Organics (Geel, Belgium). Ammonium acetate was purchased from Mallinckrodt (Phillipsburg, NJ). Polypropylene glycol (PPG) tuning solution was purchased from Varian, Inc. (Santa Clara, CA). High pressure liquid nitrogen (99.99%), zero grade air and UHP argon were purchased as compressed gas from National Welders Supply, Inc. (Morrisville, NC). Nylon membrane filters (0.45 μm) used to filter mobile phase solutions were purchased from Whatman International Ltd. (Maidstone, England).

Gas Chromatography Analysis

Solvents OmniSolve MtBE and HPLC grade hexane were purchased from Fisher Scientific (Pittsburgh, PA). Two standards of chloroform purchased from Supelco (Bellefonte, PA) were used in this study; a 5000mg/L standard for confirmation of presence and a newer 1000mg standard for calibration. Hexachlorobenzene, used as an instrument performance check standard, was also purchased from Supelco (Bellefonte, PA). Internal standard 1,2-dibromopropane was purchased from Sigma-Aldrich (St.

Louis, MO). UHP helium, UHP nitrogen and medical grade carbon dioxide were purchased as compressed gases from National Welders Supply, Inc. (Morrisville, NC).

Dissolved Organic Carbon (DOC) Analysis

The DOC calibration standard, potassium hydrogen phthalate, was purchased from Sigma-Aldrich (St. Louis, MO). Concentrated hydrochloric acid (certified ACS plus) for pH adjustment was purchased from Fisher Scientific (Pittsburgh, PA). Nylon membrane filters (0.45 μm) used for filtering samples were purchased from Whatman International Ltd. (Maidstone, England). Zero grade air was purchased from National Welders Supply, Inc. (Morrisville, NC).

Solid Phase Extraction (SPE)

Waters (Milford, MA) Oasis hydrophilic-lipophilic balance (HLB) 3 cc (60mg) SPE cartridges (Lot: W3156J4) were used for SPE. OmniSolve MtBE and HPLC grade methanol were purchased from Fisher Scientific (Pittsburgh, PA). Ethylenediaminetetraacetic acid, disodium trihydrate (Na_2EDTA 99%+) was purchased from Sigma-Aldrich (St. Louis, MO). Formic acid (98%) was purchased from Acros Organics (Geel, Belgium). UHP nitrogen was purchased from National Welders Supply, Inc. (Morrisville, NC).

2.2 Methods

2.2.1 Solid Phase Extraction

Solid phase extraction (SPE) was used to concentrate aqueous samples for analysis by LC-MS/MS. Neat CEEC stock standards were prepared at 1000mg/L in HPLC grade methanol. For CEECs in powdered form, standards were prepared by dissolving 10mg of the target CEEC in 10mL of HPLC methanol in volumetric flasks. For CEECs in liquid form, standards were prepared by calculating the volume of CEEC needed to be dissolved into 10mL of HPLC methanol in volumetric flasks from the given density. Stock standards were stored in the freezer (-20^{o}C) and used within six months of preparation. Table 2.1 shows the concentration of a typical stock solution concentration and its dilution used for the experiments. A mixture of the 6 CEECs (1° dilution) was prepared at 20mg/L by spiking 200µL of each stock solution into 10mL of HPLC grade methanol (Table 2.1). This solution was also stored in the freezer for use within six months. At different stages of the research, either caffeine or its deuterated analogue were available but only one of them was used at a time in the stock solution and its subsequent dilutions. The secondary (2°) dilution was prepared at a concentration of ~0.5mg/L by spiking 250µL of the primary (1°) CEEC mixture in 10mL of LGW. The 2° dilution was used to create a standard addition calibration curve for quantifying the

CEECs in samples, which were prepared in 250mL aliquots. The concentration of the six CEECs used in the standard addition calibration curve is shown in Table 2.2, and are added to each sample right before the addition of Na_2EDTA.

Table 2.1: An example of CEEC solution concentrations used in this study.

CEEC	Stock (mg/L)	1° dilution (mg/L)	2° dilution (mg/L)
Atorvastatin	970	19.4	0.485
Fluoxetine	1030	20.6	0.515
Caffeine-d3*	1069	21.4	0.535
Caffeine*	1093	21.9	0.548
Iohexol	1000	20.0	0.500
Tetracycline	1074	21.5	0.538
Tetrabromobisphenol A	1026	20.5	0.513

*Only one of these was present in each batch of stock solution and subsequent dilutions,

Table 2.2: Typical CEEC concentrations used for standard addition quantification.

CEEC	Cal 1 (ng/L)	Cal 2 (ng/L)	Cal 3 (ng/L)	Cal 4 (ng/L)
Atorvastatin	48.5	243	679	1067
Fluoxetine	51.5	258	721	1133
Caffeine-d3*	53.5	267	748	1176
Caffeine*	54.8	274	767	1207
Iohexol	50.0	250	700	1100
Tetracycline	53.8	269	753	1184
Tetrabromobisphenol A	51.3	256	718	1129

*Only one of these was present in each batch of stock solution and subsequent dilutions,

All standards and extracting solutions used for SPE were prepared the day of or day before the extraction including 0.25g/L Na_2EDTA stock solution in LGW and 1/9 (v/v) HPLC grade methanol/MtBE, which was prepared ahead of time by mixing HPLC grade methanol and MtBE at a 1 to 9 volume ratio and then filtered through a 0.45μm

nylon filter. The internal standard, simeton, was prepared and stored in the freezer at 1.25mg/L in HPLC grade methanol. The aqueous samples were filtered and adjusted to a pH of ~2 with formic acid. Immediately before extraction, Na_2EDTA was added at a concentration of 1mg/L to chelate the metal ions in the samples and prevent them from binding to the active sites on the solid phase cartridge which would otherwise lower analyte retention and recovery.

Extraction of the CEEC analytes used a Supelco Visiprep (Bellefonte, PA) extraction manifold. The Waters Oasis HLB 3cc SPE cartridges were preconditioned with 5mL of MtBE followed by 5mL of HPLC grade methanol and then 5mL of reagent water. Reagent water is the sample medium, such as tap water, LGW, or raw water. The sample containers were then connected to the SPE cartridges using teflon tubing and the samples extracted at a flow rate of 5mL/min. After rinsing the sample bottles, lines and cartridges with reagent water, the cartridges were dried under vacuum (15-18 Hg) for 30 minutes to remove excess water. Sample elution was performed using 5mL of HPLC grade methanol followed by 5mL of 1:9 HPLC grade methanol/MtBE. The extract was collected in 15mL clear glass conical vials and then concentrated under UHP nitrogen using a Pierce (Rockford, IL) Reacti-Vap Model 18770 to a volume of about 200µL. Extracts were brought to a final volume of 250µL using HPLC grade methanol. 10µL of internal standard simeton in HPLC grade methanol was then added to the extract using a 10µL glass syringe to produce a final concentration of 50µg/L. The sample extracts were then vortexed using a Thermolyne type 16700 mixer (Dubuque, IA) to ensure

complete mixing of the internal standard. Sample extracts were stored in the freezer at -20^{o}C for no more than 7 days before analysis.

2.2.2 Analytical Methods

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

The LC-MS/MS instrumentation consists of a Varian 210 ProStar Solvent Delivery Module (Walnut Creek, CA) with a Metachem Technologies, Inc. Degassit Unit (Torrance, CA), a Varian 430 ProStar Autosampler (Walnut Creek, CA) and a Varian 1200L Triple Quandrupole Mass Spectrometer (Palo Alto, CA) with electro spray ionization (ESI) or atmospheric pressure chemical ionization (APCI) chamber. The CEECs were separated by a Varian Pursuit C-18 guard (3cm X 2mm, 3μm) and analytical (15cm x 2mm, 3μm) column (Walnut Creek, CA). There were two mobile phases; mobile phase A contained 0.1% (v/v) acetic acid and 0.2% (w/v) ammonium acetate in LGW while mobile phase B was 100% HPLC grade methanol. Both were filtered to 0.45μm prior to use and stored in 1L glass bottles. For separation of the CEECs, the mobile phase was programmed according to Table 2.3.

Table 2.3: Mobile phase program used for LC-(+/-)-ESI-MS/MS (flow rate: 0.2mL/min)

Time	% Mobile Phase A	% Mobile Phase B
0 min	90	10
30 sec	90	10
35 sec	50	50
8 min	0	100
20 min	0	100
21 min	90	10
26 min	90	10

The MS/MS settings for each target CEEC were optimized by infusing a 1 mg/L solution in 11/9 (v/v) MtBE/methanol, which is the extract solution ratio from SPE without blow down (Vanderford & Snyder 2006), of each CEEC individually at a flow rate of 20μL/min delivered by a Harvard Apparatus Syringe Pump (Holliston, MA). The target CEECs were analyzed in two different ionization modes; positive (+) ESI and negative (-) ESI. The different ion modes used different ionization gases; (+) ESI used UHP argon while (-) ESI used zero grade air. The parameters for the MS/MS are listed in Table 2.4. The drying and nebulizing gas was high pressure liquid nitrogen (99.99%). The collision energy for second quadruple (Q2) varied from 0 to 50 eV. The daughter ion intensity was plotted as a function of the collision voltage on the MS/MS breakdown curves recorded by the Varian Workstation 6.3 software. The ionization modes, parent ion, daughter ions, retention time, and collision energy of the ions of each CEEC are shown in Table 2.5.

Table 2.4: Source dependent mass spectrometer parameters

Parameter	Set Value
Nebulizer Needle Voltage	5000V
Shield Voltage	600V
Detector Voltage	(+) ESI:1445V (-)ESI:1500V
Drying Gas Flow Rate	18 psi
Nebulizer Gas Flow Rate	55 psi
Collision (CID) Gas Flow Rate	2.2 mTorr
Chamber Temperature	50°C
Drying Gas Temperature	300°C

Table 2.5: Parameters for CEEC analysis the study

	Ionization mode	Retention time (min)	Parent ion m/z (collision energy, (V))	Daughter ion 1 m/z (collision energy, (V))	Daughter ion 2 m/z (collision energy, (V))
Atorvastatin	(+)ESI	7.7	581.4 (5.5)	488.4 (22)	519.5 (26.5)
Fluoxetine	(+)ESI	8.3	310.0 (5.0)	44.1 (5.5)	148.1 (5.0)
TBBPA	(-)ESI	8.0	542.7 (5.5)	417.7 (35.5)	445.7 (28.0)
Iohexol	(+)ESI	7.7	822.0	N/A	N/A
Tetracycline	(+)ESI	6.2	445.3 (6.0)	154.0 (18.5)	410.0 (15.0)
Caffeine-d3	(+)ESI	5.3	198.1 (5.0)	141.0 (11.0)	113.0 (15)
Caffeine	(+)ESI	5.3	195.1 (6.0)	138.0 (13.0)	109.6 (17.5)
Simeton	(+)ESI	8.3	198.1 (5.0)	123.0 (10.0)	N/A

N/A=not available

Dissolved Organic Carbon (DOC)

DOC analysis used a Shimadzu Corp. TOC-VCPH Total Organic Carbon Analyzer with ASI-V Auto Injector. All water samples were filtered through 0.45µm nylon filters as a pretreatment for analysis. The DOC calibration stock standard was potassium hydrogen phthalate at a concentration of 1000mg/L as C which was used within two months of preparation. The DOC working solution was a dilution of the DOC stock standard to 100mg/L as C and prepared weekly to create a calibration curve

for analysis. Calibration points were at concentrations of 0, 0.05, 0.5, 5, and 10mg/L as C. Before analysis, pH adjustment to 2 was achieved by addition of 8 drops of 2N hydrochloric acid, prepared from concentrated hydrochloric acid, to each 40mL aliquot. The samples were then put on the instrument for analysis. Multiple LGW samples and one performance check standard at 5mg/L C were run before the calibration curve, which were followed by samples. After every 10 samples, a performance check standard was included in the autosampler sequence.

Total Organic Halogen (TOX)

A series of treatments are needed before samples are ready for TOX analysis. Samples were collected in 125mL amber bottles and if they contained free chlorine were quenched with an appropriate amount of sodium sulfite depending on the concentration of chlorine residual. The concentration of quenching agent required was calculated by the following equation;

$$\text{chlorine mg/L} \times 3.54 = \text{mg/L sodium sulfite needed}$$

The value 3.45 is the molar ratio between chlorine and sodium sulfite multiplied by a safety factor of 2 to ensure complete quenching. Samples were stored headspace-free at 4^{o}C for no more than 14 days were brought to room temperature right before analysis. Samples were pH adjusted to 2 (approximately 1 drop of concentrated sulfuric acid was added to each 125mL sample) and checked with pH paper to ensure that all the organic analytes are in the protonated form for better absorption onto carbon. A

volume of the sample was then absorbed onto a carbon column using a Tekmar-Dohrmann AD-2000 Adsorption Module (Cincinnati, OH) at a flow rate of 2mL/min followed by a 2mL nitrate rinse (1.13g KNO_3/L as N) at 1mL/min to remove adsorbed inorganic halogen ions. The carbon columns were wrapped in aluminum foil if not immediately injected onto the Rosemount Dohrmann DX-2000 Organic Halide Analyzer (Cincinnati, OH) but not held for more than 6 hours. The volume of sample absorbed onto the column was determined by estimating the total amount of organic halogen in μg of the sample so that it would fall into linear range of the detection for the instrument. Nitrate rinse blanks, which were samples of LGW of the same volume as the sample and taken through the nitrate rinse on a clean carbon column, were run at the beginning and end of adsorption as a background control for each batch of samples.

The Organic Halide Analyzer was used to analyze the carbon columns. The instrument used oxygen for combustion and UHP helium as a carrier gas. The scrubber vial was attached as the instrument was started up and detached when all sample analyses were complete. The scrubber vial was filled with 80% (v/v) sulfuric acid. The coulometric cell was conditioned as needed with acetic acid and silver acetate. The furnace temperature was set at 850^{o}C. A cell check was carried out before sample injection by spiking 5μL of 200ngCl/μL NaCl solution into the cell; 90-110% recovery was required to proceed with the next step, a combustion check in which 5μL of 500ng/μL of 2,4,6-trichlorophenol in HPLC methanol was spiked on top of a dry carbon column. 90-110% recovery was required to proceed with sample analysis.

Gas Chromatography (GC)

The Hewlett Packard 5890 GC with an electron capture detector (ECD), a Hewlett Packard GC/SFC 7673 Injector, and Hewlett Packard 7673 Controller were used to search for and quantify volatile halogenated byproducts. The separation column was a Phenomenex (Torrance, CA) ZB 1MS (30m x 0.25mm I.D. x 1.0μm Film Thickness). The temperature program is shown in Table 2.6. The injector temperature was set at 250^{o}C and sample injection (splitless-split) volume was 1μL. The temperature of the detector was set at 300^{o}C. A hexachlorobenzene (HCB) solution in hexane at 100μg/L was run at the beginning and end of a set of samples as a performance check for the instrument. The carrier gas was UHP helium at a flow rate of 1mL/min.

Table 2.6: Temperature program for GC-ECD

Time	**Temperature (^{o}C)**
0 min	35
10 min	35
36.5 min	300
40 min	300

The GC paired with mass spectrometry (MS) used a Varian Saturn 2200 ion trap with the Varian CP-3800 Gas Chromatograph to identify volatile byproducts from the reaction of disinfectants with CEECs. The separation column used was a Phenomenex ZB 5MS (30m x 0.25mm I.D. x 0.25μm Film Thickness). The temperature program was that shown in Table 2.6. The filament was turned on 3.25 minutes after sample injection to avoid solvent front overload. The injector temperature was set at 250^{o}C and a sample

extract volume of 1μL was injected manually. Before the first sample injection, an air/water check was carried out to ensure that there was no leak in the system. HCB in hexane (100μg/L) was used as a performance check before any sample run and to ensure instrument sensitivity by comparing the area and height of the peak to those obtained in previous runs. The detector was set to scan the mass range of 70-150 Daltons in positive ionization mode. The carrier gas was UHP helium at a flow rate of 1mL/min.

Ultraviolet-Visible (UV-Vis) Analysis

UV-Vis characterization of samples was undertaken using a Hitachi U-3300 Spectrophotometer (Danbury, CT). The solutions and standards prepared at 10mg/L in LGW were scanned from 200-700nm to determine the wavelength of maximum absorption (λ_{max}).

Medium Pressure (MP) UV System

The bench-scale MP-UV system was used to stimulate the UV treatment process in water treatment plants. The samples were treated in a custom built unit (Figure 2.1) with a 500W MP-UV lamp (Ace-Hanovia, Vineland, NJ). The sample was contained in a 100mL Pyrex crystallization dish with a copper coil around it connected to a Coolflow CFT-33 refrigerated recirculator (NESLAB Instruments, Inc. Newington, NH) set at 18^{o}C to keep the sample at a constant temperature during treatment and stirred constantly. A manual shutter was used to start and stop the UV treatment. Before using the MP-UV

system to treat any samples, the irradiance of the lamp was evaluated with actinometry using a 0.012mM uridine solution prepared from dilution of 1mL of uridine stock (12mM) and 10mL of phosphate buffer (100mM at pH 7) into 1 L of LGW, which can be used for six months stored at 4°C. The uridine solution was brought to room temperature before irradiating. The volume of the uridine solution irradiated should be the same volume as the amount of sample that is treated. The uridine solution was irradiated for a set period of time (x minutes) after the MP-UV lamp has warmed up for 30 minutes. A sample of untreated uridine solution was set aside for actinometry calculations (designated as t=0). The t=0 and irradiated samples (designated as t=x) were analyzed using a Hitachi U-3300 Spectrophotometer (Danbury, CT) from 200-400nm. Along with the uridine samples, the samples prepared to be UV treated were also analyzed with the spectrophotometer for their absorbance in this range. The time for irradiation was then calculated based on these absorbance values to achieve the required UV dose (mJ/cm^2) using the "MP UV dose calculation" spreadsheet (Lyon 2012). The samples were then irradiated for the calculated period of time. The uridine actinometric test was carried out at the end of one day's batch of samples if the lamp has operated for more than 12 hours.

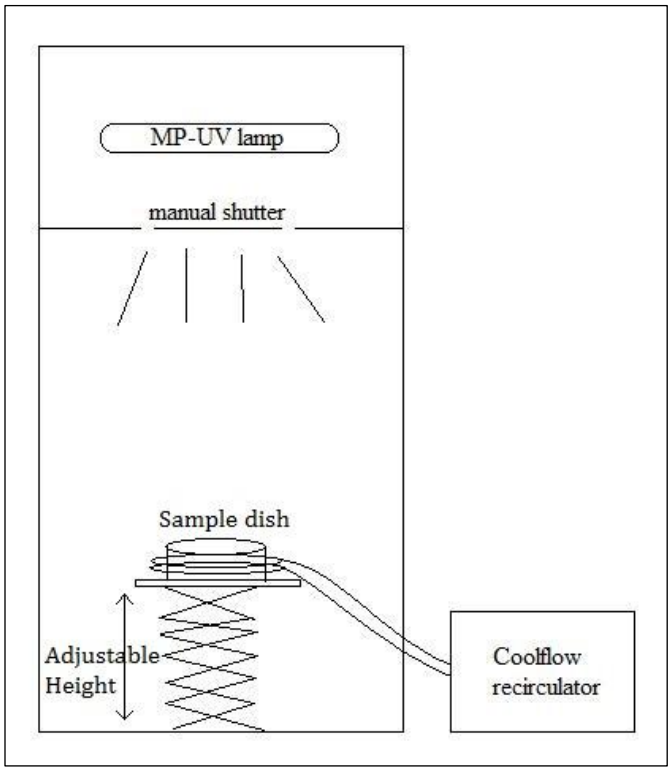


Figure 2.1: Diagram of the MP-UV system.

2.2.3 Bench Scale Coagulation Experiment

Raw water samples were collected from OWASA (Carrboro, NC), which has two sources of water, University Lake and Cane Creek Reservoir (Orange County, NC). The utility uses a combination of two waters as the source for treatment. On the day of collection the utility used a mix of 85% Cane Creek with 15% University Lake water, which had a pH of 6.55 and turbidity of 1.09NTU. The raw water samples used in this study were collected from Cane Creek Reservoir and had a DOC concentration of 2.93mg/L as C, pH of 5.6 and a turbidity of 1.52NTU. The water samples were collected in one 2.5L and seven 4L amber glass bottles. Samples were transported

immediately to the environmental chemistry laboratory in the Michael Hooker Research Center at UNC and immediately placed in a refrigerator at 4°C and used within two weeks of collection.

Preliminary jar test experiments determined the optimum dose of coagulants and amount of pH adjustment needed; all jar tests used the Phipps & Bird Inc. (Richmond, VA) Laboratory Stirrer (110 Volts AC). The coagulants were aluminum sulfate and ferric sulfate and prepared as stock solutions in LGW at 7500mg/L as aluminum sulfate hydrate and ferric sulfate hydrate. The OWASA raw water was combined and mixed in a 20L glass container with a magnetic stirrer for uniform water quality. The raw water was then separated into two 2L rectangular pyrex jars by measuring out 1.5L of the raw water with a graduated cylinder. The pH of the raw water was tested as the coagulants were added to the water while mixing by increments (Table 2.7) of coagulant concentration. Concentrations for aluminum sulfate were 20, 30, 40, 60 mg/L and 40, 50, 60, 70, 80 mg/L for ferric sulfate. The pH was adjusted for each coagulant concentration with either 0.1N sodium hydroxide or 0.2N sulfuric acid to reach the target pH of 5.6 and 5.8 (Knappe et al. 2012) for aluminum sulfate and ferric sulfate, respectively. 0.1N sodium hydroxide was prepared by dissolving 4g of sodium hydroxide pellets in 100mL of LGW then filtered to 0.45μm using nylon filters. 0.2N sulfuric acid was prepared by adding 100μL of concentrated sulfuric acid into 100mL of LGW in volumetric flasks.

Table 2.7: Coagulant concentration and stock added

Aluminum sulfate concentration (mg/L)	**mL of aluminum sulfate stock added to 1.5L sample**	**Ferric sulfate concentration (mg/L)**	**mL of ferric sulfate stock added to 1.5L sample**
20	4	40	8
30	4+2	50	8+2
40	4+2+2	60	8+2+2
50	4+2+2+2	70	8+2+2+2
60	4+2+2+2+2	80	8+2+2+2+2

A second preliminary jar test determined the optimum coagulant concentration and settlement time by using various concentrations (aluminum sulfate: 20, 30, 40, 60 mg/L, ferric sulfate: 40, 50, 60, 70, 80 mg/L) of coagulant added to different jars and the corresponding volume of acid or base for pH adjustment needed for each dose was added before the coagulant. The mixing conditions for the jar test were 30 seconds of rapid mix at 100 rpm followed by 36 minutes of flocculation at 25 rpm and settlement for 10, 30, and 60 min. The turbidity of the water was tested before pH adjustment and after coagulation and settlement time to determine conditions for maximum removal of turbidity.

In the experiment to evaluate the effect of coagulation on CEECs, 1 to 2 L aliquots of raw OWASA water were spiked with a 1° dilution mixture of CEECs (Table 2.1) at a concentration of 1000ng/L in volumetric flasks. This 1° dilution mixture included caffeine-d3. All the spiked samples were then mixed together using a magnetic stirrer in a 20L glass container to create a uniform solution. The sample was then separated into six jars by measuring out 1.5L of the raw water with a graduated cylinder. Four jars

were treated with either aluminum sulfate or ferric sulfate according to results from the previous experiments. The treated and untreated samples were then taken through SPE and analyzed by LC-MS/MS. Quality control samples for each coagulant were created by spiking 250ng/L of the CEEC mix into coagulated and settled water samples after filtering through 0.45μm nylon filter. A sample of unspiked raw water, spiked raw water, aluminum sulfate-treated water, and ferric sulfate-treated water were tested for DOC, turbidity, and pH.

2.2.4 Bench Scale PAC Experiment

The surface water described in section 2.2.3 was also used in these tests. The raw water was spiked with the 1° dilution mixture of CEECs (Table 2.1) at a concentration of 1000ng/L in 2L and 1L volumetric flasks, which included caffeine in place of caffeine-d3. All the spiked samples were mixed together using a magnetic stirrer in a 20L glass container. Two aliquots of the spiked raw water were withdrawn into 250mL amber bottles as controls and filtered through 1.5μm glass micofibre filters. Three PAC doses of 5, 10 and 20 mg/L were tested and each mixed in a 4L amber bottle using a magnetic stirrer and Cimarec 2 stir plate (Mastead/Themolyne, Dubuque, IA). To prepare the PAC, it was oven dried at 180^{o}C to constant weight for about 24 hours and each PAC dose was weighed out and added to samples with a few drops of LGW.

250mL samples were pulled out after mix times of 2, 5, 10, 15, 30, 60 and 120 minutes and were filtered immediately through 1.5μm glass micofibre filters to end the interaction between PAC and the water. For quality control, 250mL aliquots were removed for each PAC dose at reaction times of 15 and 60 minutes and 250 ng/L of CEEC mixture was spiked in. Standard addition calibration samples used the 10mg/L PAC dose sample pulled after 15 minutes. The treated and untreated samples were taken through SPE and then analyzed by LC-MS/MS.

2.2.5 Chlorination and UV Byproduct Identification

Individual CEEC solutions were prepared at 10mg/L in LGW and separated into three samples; one for chlorination, one for MP-UV treatment, and one for a control sample (untreated sample). A single MP-UV treatment dose of 1000mJ/cm^2 was used to generate detectable changes. The chlorination treatment used a chlorine to CEEC molar ratio of 20:1 and samples were reacted for 24 hr in the dark under headspace-free conditions at 20°C in 20mL amber bottles covered with foil. An example calculation for chlorine dose using tetracycline (molecular weight of 444.4) is shown here.

$$\frac{10\text{mg } tetracycline}{L} \times \frac{1g}{1000mg} \times \frac{1mol}{444.4g} = 2.25 \times 10^{-5} moles/L \; T\text{etracycline}$$

$$\frac{2.25 \times 10^{-5} moles}{L} T\text{etracycline} \times 20 = 450 \mu moles/L \; Cl_2$$

$$\frac{450 \mu moles \; Cl_2}{L} \times \frac{1mol}{1000000 \mu moles} \times \frac{71g}{1mole} \times \frac{1000mg}{1g} = 31.9 mg/L \; Cl_2$$

After 24 hours reaction, the chlorinated samples were quenched with ascorbic acid according to the free chlorine residual concentration. The ascorbic acid was added as a solution in LGW and the amount needed calculated using the equation below;

$$\text{chlorine mg/L} \times 4.96 = \text{mg/L ascorbic acid needed}$$

The value 4.96 is the stoichiometric molar ratio between chlorine and ascorbic acid multiplied by a safety factor of 2 to ensure complete quenching. Table 2.8 shows the chlorine dose used for each CEEC. Treated samples and controls were then directly infused onto the MS/MS at a flow rate of 20μL/min delivered by a Harvard Apparatus (Holliston, MA) Syringe Pump. Product ions were identified by scanning mass to charge (m/z) every 100 Daltons from 50 to 900 Daltons using the same MS conditions listed in Table 2.4. The treated samples were compared to the control sample of 1mg/L of CEEC in LGW scanned in the same m/z range. Ions with significant signal that were absent in the control sample were recorded and daughter ions for each product ion were identified if they were generated. From this information, product structure was hypothesized.

Table 2.8: Chlorination dose for each 10mg/L CEEC reaction

Compound	Chlorine dose (mg/L)
Atorvastatin	23.5
Caffeine-d3	72.7
Fluoxetine	41.0
Iohexol	17.3
Tetracycline	31.9
TBBPA	26.1

2.2.6 Chlorination of Tetracycline Experiment

In a specific case study, the UV-Vis absorbance of the ascorbic acid-quenched, chlorine-treated tetracycline (10mg/L) was compared to the untreated sample using a Hitachi (Danbury, CT) U-3300 Spectrophotometer. Another sample of tetracycline was prepared at a higher concentration (100mg/L) and reacted with chlorine again at a 20:1 molar ratio under the same reaction conditions and quenching as described in Section 2.2.5. 20mL samples aliquots were removed and extracted with 4mL of MtBE. The extracts were analyzed on the GC-ECD and GC-MS for volatile halogenated byproducts.

A separate kinetic experiment was performed to determine the rate of formation of byproducts from 1mg/L tetracycline reacted with 5mg/L chlorine (molar ratio 1:31.3) for 24 hours at two different pH values (7 and 9) in LGW verified with a Thermo Scientific Orion 3 Star pH benchtop pH meter and Accumate pH probe (Cat # 13-620-108A). Samples were separated into 125mL and 40mL amber bottles headspace-free and reacted separately. The 125mL samples were used for TOX analysis and the 40mL samples were used for GC-ECD analysis. The samples were chlorinated separately for 10 sec, 20 sec, 30 sec, 1 min, 2 min, 5 min, 10 min, 20 min, 30 min, 60 min, 2 hrs, 3hrs, and 24 hrs and the reaction quenched with sodium sulfite (for TOX) and ascorbic acid (for volatile halogenated byproducts). During longer reactions, samples were placed in the dark, headspace-free. The amount of quenching agent needed was calculated for 5mg/L of chlorine and tested beforehand by confirming zero free chlorine when added to different

chlorine residuals measured with the HACH (Loveland, CO) test kit pocket colorimeter and HACH permachem DPD free chlorine reagents. The amount of quenching agent added was 24.8mg/L for ascorbic acid and 17.7mg/L for sodium sulfite for all samples. The concentration of quenching agent required was calculated by the equations described previously for ascorbic acid and sodium sulfite. 20mL of the 40mL samples were removed from their vials and extracted with 4mL of MtBE spiked with internal standard 1,2-dibromopropane (100μg/L). The extracts were analyzed using the GC-ECD method described in section 2.2.2. The 125mL samples were analyzed for TOX using 50mL of the sample for absorption onto the carbon columns as described in section 2.2.2.

2.2.7 Comparison of Chlorination and Chloramination of Tetracycline

A tetracycline stock solution was prepared at a target concentration of 1000mg/L in HPLC grade methanol from which a dilution at 1mg/L in LGW was used for chlorination and chloramination at a 20mg/L as Cl_2 dose. Monochloramine solution was prepared ahead of time by adding free chlorine drop-wise to 24 mM ammonium chloride solution (adjusted to pH 8.5 with sodium hydroxide) at 1.2:1 N:Cl molar ratio (See Appendix A for Standard Operating Procedure). The reactions between 1mg/L of tetracycline and 20mg/L disinfectant occurred for 24 hr in the dark under headspace free conditions at 20°C in a 125mL amber bottle covered with foil. The solution was quenched

with sodium sulfite after determining the free and total chlorine residual concentration with HACH test kit pocket colorimeter and HACH permachem DPD free chlorine reagents. The samples were analyzed for TOX using 50mL of the sample for absorption onto the carbon columns as described in section 2.2.2.

3. RESULTS AND DISCUSSION

3.1 Solid Phase Extraction Recovery (SPE)

The recovery of each target CEEC from SPE or as a result of matrix effects was evaluated using LC-MS/MS through parent ion count, area, and relative area (area of CEEC/area of internal standard) as described in Section 2.2.1 and 2.2.2. The samples evaluated were a standard at 1mg/L CEEC mixture in 9/11 methanol/MtBE, three samples of OWASA raw water (RW) SPE extract spiked at different CEEC concentrations (0.25mg/L, 0.5mg/L and 1mg/L), a sample of 1mg/L CEEC mixture spiked into raw water before filtration pretreatment (RWB), and a sample of 1mg/L CEEC mixture spiked in to raw water after filtration pretreatment (RWA). Tetracycline, which did not fulfill quality control criteria, was not included in the data analysis due to poor signal response and high background noise. None of the targeted CEECs were detected in the unspiked raw water. In order to evaluate whether the detector signal response was affected by the raw water matrix, the chromatographic peak areas (Figure 3.1) and parent ion counts (Figure 3.2) for the 1mg/L standard mix and raw water extract spiked with 1mg/L standard mix were compared side by side. Table 3.1 shows that the

signal of caffeine is most impacted by the raw water extract matrix in both ion count and area. Relative area could not be used for assessment due to the suppressed signal of the internal standard, simeton, by the extract matrix.

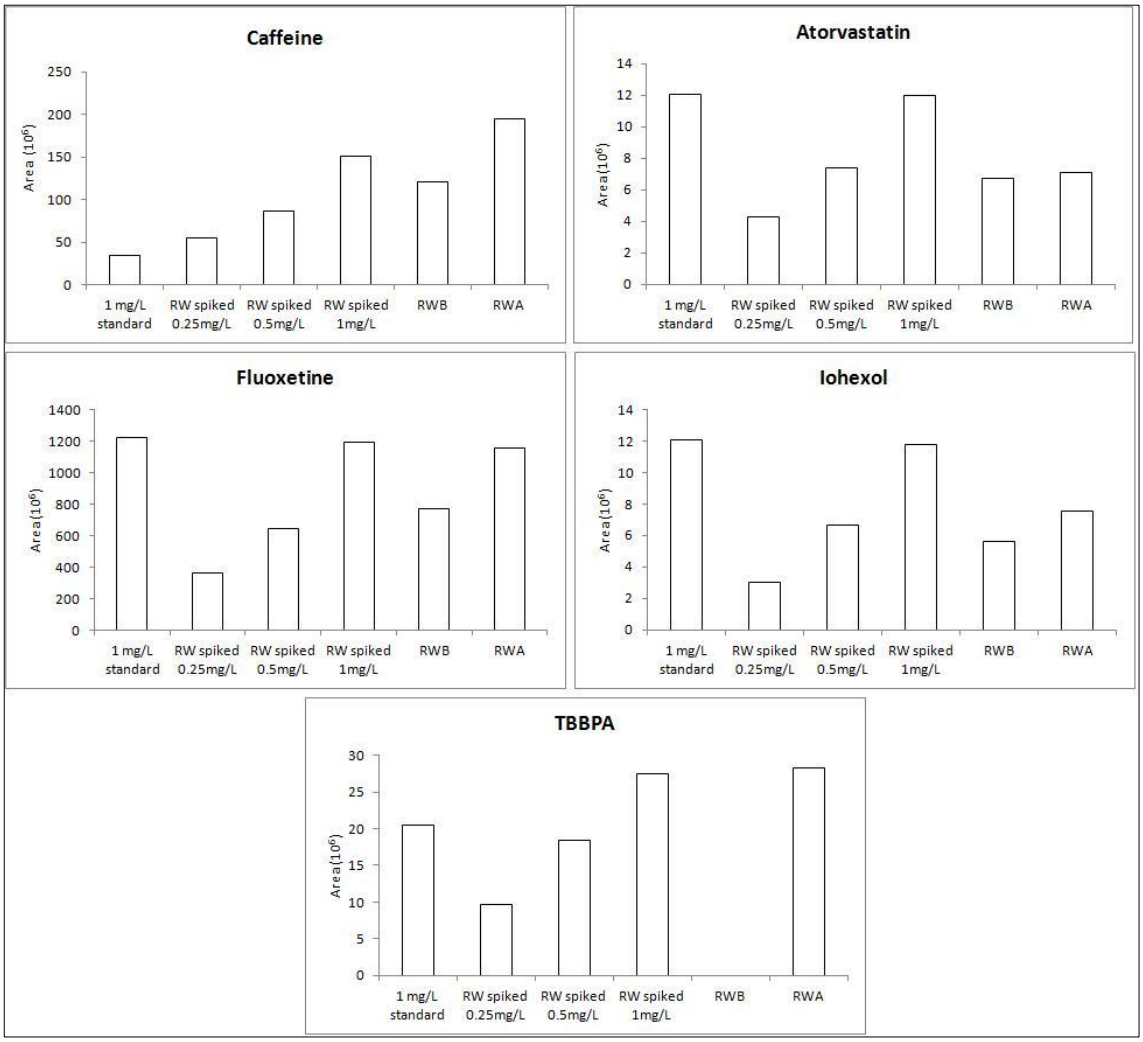


Figure 3.1: Comparison of LC-MS/MS area response for each CEEC in the SPE extract (n=1). RW = raw water, RWB = raw water spiked before filtering, RWA = raw water spiked after filtering.

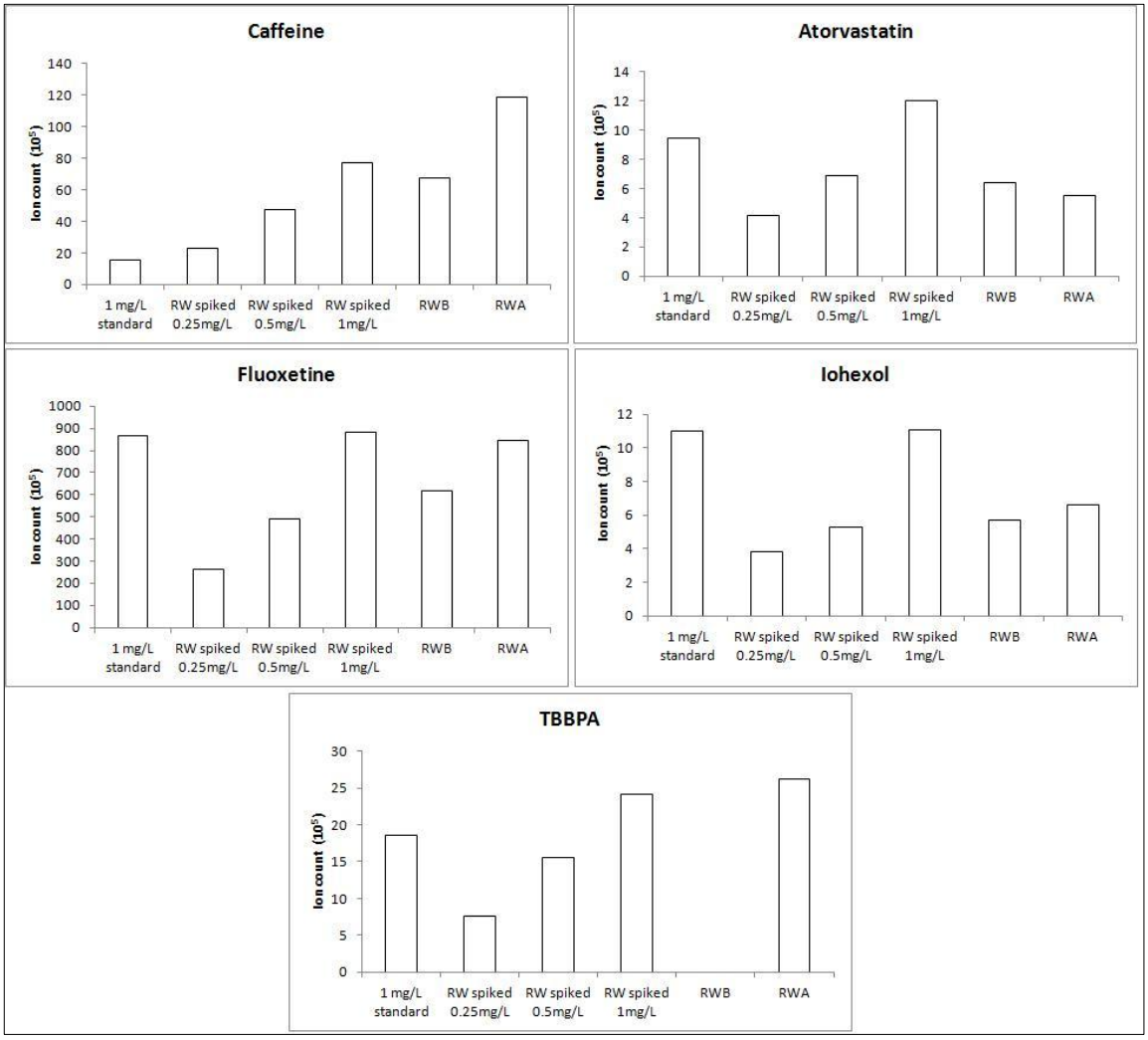


Figure 3.2: Comparison of LC-MS/MS ion count response for each CEEC in the SPE extract (n=1). RW = raw water, RWB = raw water spiked before filtering, RWA = raw water spiked after filtering.

Table 3.1: Effect of raw water extract on signal response calculated by area and ion count (comparing 1mg/L standard and 1mg/L extract spike).

	Caffeine	**Fluoxetine**	**Atorvastatin**	**Iohexol**	**TBBPA**
Raw Water + 1mg/L extract spike (area)	434%	98%	99%	97%	130%
Raw Water + 1mg/L extract spike (ion count)	491%	102%	127%	100%	130%

All CEECs in the RWA sample had a higher signal than in the RWB sample when comparing both ion count (Figure 3.2) and area (Figure 3.1), which suggests that the CEECs may have been filtered out with the suspended solids in the raw water or have adsorbed onto the nylon filter during the pretreatment for SPE. This is particularly the case with TBBPA, which was not detected when spiked into raw water before filtering but is fully recovered when spiked into raw water after filtration (Table 3.2). This could be due to the high log Kow of TBBPA, which is 4.50.

CEEC recovery from SPE was calculated by dividing the response from the RWA or RWB samples by that for the 1mg/L CEEC mixture raw water extract spike (Table 3.2). The 1mg/L extract spike takes into account the signal enhancement from the raw water matrix for more accurate analysis. Recovery was not analyzed using relative area because internal standard was not added to RWA and RWB. Among the target CEECs, caffeine had the highest recovery while iohexol had the lowest. Comparing analyte recovery in the spiked filtered raw water samples, atorvastatin and iohexol had the lower recoveries from SPE extraction relative to caffeine and TBBPA, which have recoveries higher than 100%. In general, analyte recovery in spiked filtered raw water samples was around the same percentage or a little higher than in the filtered spiked raw water sampled for all compounds except TBBPA, which was lost during filtration as already described. Since TBBPA is lost during filtration, there would be little difference if standard addition for calibration occurs before or after filtration of the samples. The current method does this after filtration and clearly cannot be used for quantifying TBBPA.

Table 3.2: SPE recovery of CEECs calculated by area and ion count (comparing RWA or RWB and 1mg/L raw water extract spike). RWB = raw water spiked before filtering, RWA = raw water spiked after filtering.

	Caffeine	**Fluoxetine**	**Atorvastatin**	**Iohexol**	**TBBPA**
RWB (area)	80%	65%	56%	48%	0%
RWB (ion count)	87%	70%	53%	52%	0%
RWA (area)	129%	97%	59%	64%	103%
RWA (ion count)	154%	96%	46%	60%	108%

A test was then carried out to determine if TBBPA was filtered out by the 0.45μm nylon filter or have attached onto the sediment which was filtered out. A sample of TBBPA in LGW was filtered with the nylon filter and analyzed for TBBPA after mixing 1:1 with HPLC grade methanol through direct infusion mass spectrometry (MS) but TBBPA was not detected. Other types of filters were then evaluated in the same manner. The 0.7μm glass-fibre filter was found to be the only one that did not filter out TBBPA, and so was used in later experiments in place of the nylon filters but, unfortunately, not prior to those described in the next section.

3.2 Coagulation for Removal of CEECs from Surface Water

The optimum coagulant dose of alum and ferric sulfate necessary for maximum removal of suspended solids in surface water was determined using turbidity

measurements before performing the bench scale study involving the CEECs according to Section 2.2.3. The pH was tested and adjusted to 5.6 and 5.8, respectively, for each dose as described in Section 2.2.3. From Figures 3.3 and 3.4 the optimum doses are 50mg/L and 70mg/L, respectively, with a settlement time of 60 minutes. Although doses of 30mg/L (alum) and 50mg/L (ferric sulfate) could also have been selected, they most likely represent the charge neutralization phase (all negatively charged colloids neutralized by positively charged coagulants and precipitates). The selected doses, however, are those for sweep flocculation, commonly used in drinking water treatment, where contaminants are swept down by the settling flocs.

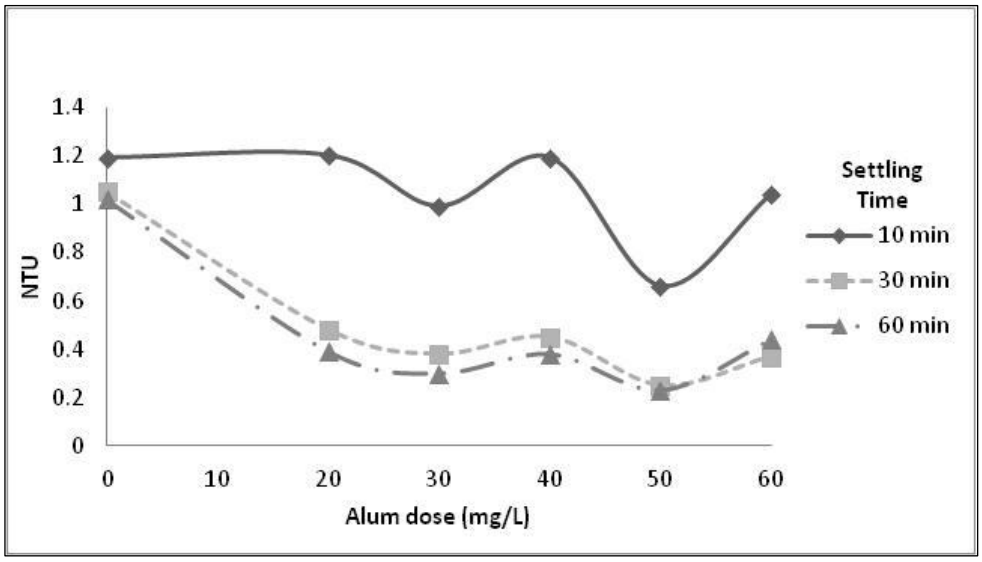


Figure 3.3: OWASA surface water turbidity as a function of varying alum dose after different settling times.

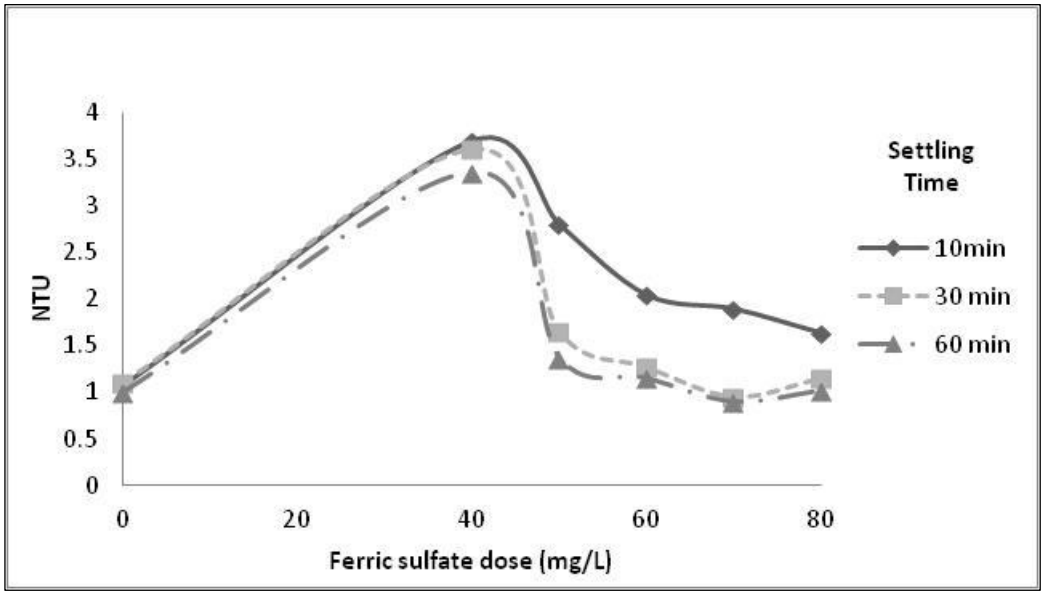


Figure 3.4: OWASA surface water turbidity as a function of varying ferric sulfate dose after different settling times.

Figure 3.5 shows the results of quality control (QC) tests which for each sample represents the extracted concentration of the target CEEC in the water to which a 250ng/L CEEC mixture spike had been added after sample filtration but prior to SPE. The "before Treatment" QC samples contained 1000ng/L CEEC in the raw water before the 250ng/L QC mix was added after filtration. The concentrations shown in figure 3.5 were obtained from a calibration curve produced for a range of CEEC concentrations in the water before filtration so that Figure 3.5 can be used to illustrate CEEC recovery from filtration. For the other samples, a similar recovery can be calculated by comparing the results in Figure 3.5 with those in Figure 3.6 which shows the impact of coagulation on CEEC removal.

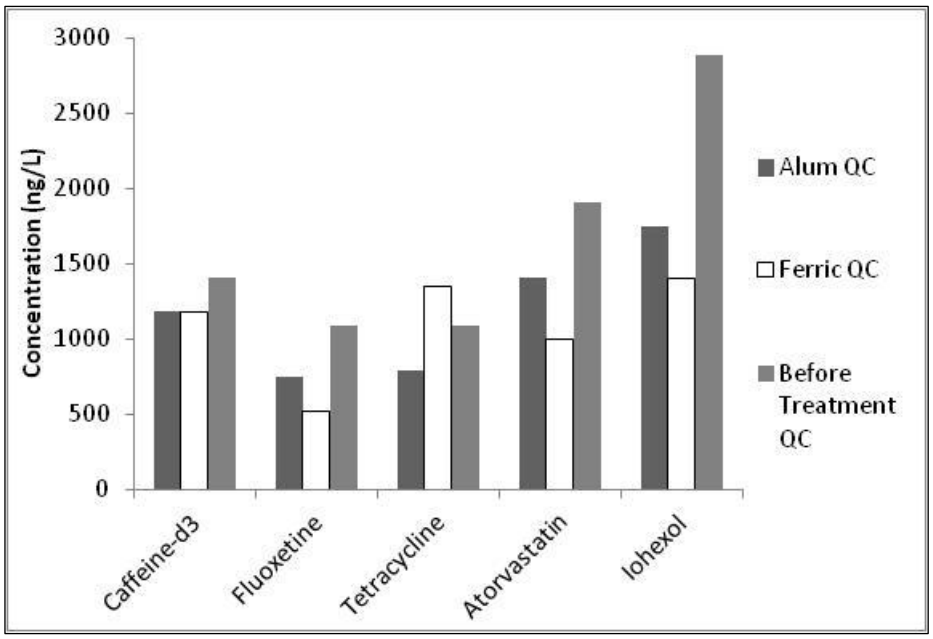


Figure 3.5: Comparison of CEEC concentration of quality control samples (250ng/L spike).

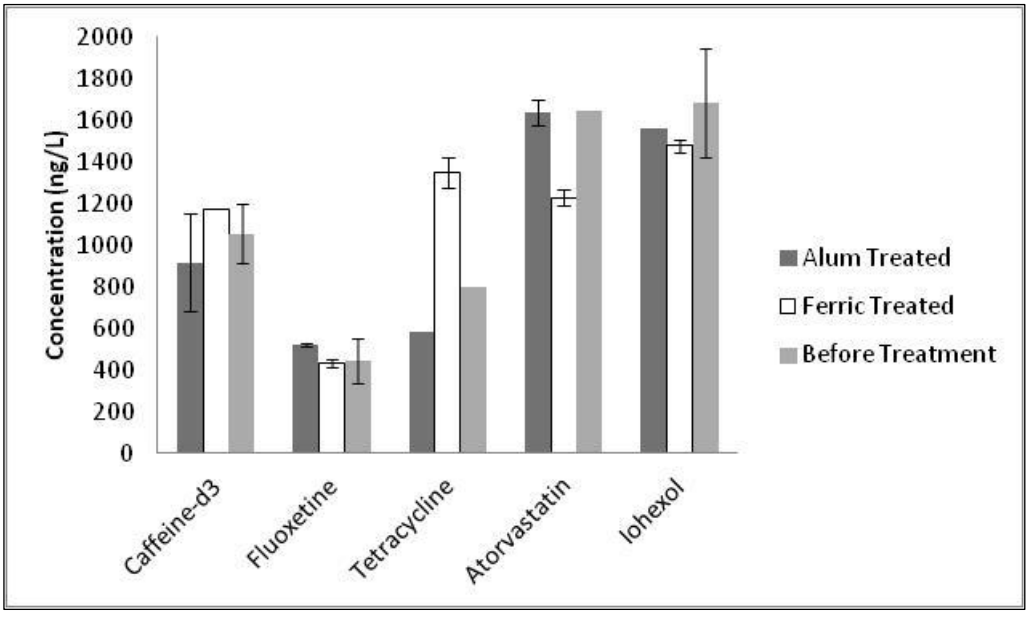


Figure 3.6: Comparison of concentration of CEEC before and after coagulation with alum and ferric sulfate. Error bars represent the difference in concentration of duplicate samples.

Overall it appears that coagulation with either alum or ferric sulfate had little to no effect on the CEECs except in the case of ferric sulfate on atorvastatin. The latter has a

pK_a value of 4.46 and thus exists as a negatively charged species in this water permitting its charge neutralization or partial bonding with NOM that aids in its removal with the flocs. The data showed two anomalies: (i) iohexol in the "before treatment" QC sample suggested a 140% recovery (see Appendix Table B-5) which would then lower the value in Figure 3.6 to below the concentration found in the coagulated samples. This should be interpreted as meaning that coagulation had no impact on iohexol; (ii) the ferric sulfate-treated tetracycline showed no recovery in the QC sample (i.e. no difference in concentration of tetracycline between the unspiked and spiked samples). The SPE method calls for addition of 1mg/L EDTA to chelate residual free cations that may otherwise complex charged target ions such as the zwitterion forms of tetracycline in this matrix and lead to unpredictable recoveries from SPE. This amount of EDTA can chelate a maximum of 0.19mg/L of ferric ion, a small amount compared to that likely to remain in the solution after coagulation and settling. A further complication will arise from any eluted ferric ions in the extract placed on the mass spectrometer which can cause signal enhancement -- a likely explanation for the elevated tetracycline signal shown in Figure 3.6. Hence, it is not possible to interpret the results for tetracycline in this experiment. Moreover, Westerhoff et al. (2005) suggest that coagulation removal is correlated with log K_{ow} in which chemicals with a higher log K_{ow} value have higher removal. This suggests that tetracycline should not be removed by coagulation. Knappe et al. (2012) reported a 74% removal of tetracycline during coagulation with ferric sulfate using similar conditions although their raw water DOC concentration was

5.2mg/L while in this study the DOC concentration was 2.9mg/L. The higher DOC may have facilitated the removal in the Knappe et al. (2012) study. Both studies showed ineffective removal of caffeine.

3.3 CEEC Removal from Surface Water by Powdered Activated Carbon (PAC)

Results for only three of the target CEECs (caffeine, fluoxetine and TBBPA) are presented because data for atorvastatin, iohexol and tetracycline did not meet quality control criteria. Figures 3.7 to 3.9 illustrate the kinetics of PAC doses 5mg/L, 10mg/L, and 20mg/L, respectively, and Table 3.3 lists the first order kinetic rate of adsorption of the CEECs. At all doses of PAC, CEEC removal is above 70% after 120 minutes of contact time; however, at the 5mg/L PAC dose there was an inconsistent trend which may be due to the CEECs and NOM adsorbing and desorbing over time causing fluctuation of dissolved CEEC concentration. The kinetic rate of adsorption increases with PAC dose except for fluoxetine which has a higher rate at 10mg/L. In general, caffeine and fluoxetine have a higher removal and rate when compared to TBBPA at the 10mg/L PAC dose. The mechanism of PAC adsorption involves the attraction between CEEC and the carbon particles through Van der Waals forces or the partitioning of CEEC between the liquid (water) and carbon (solid) phase. The affinity of each CEEC to PAC cannot be predicted based on log Kow. At each contact time, the removal of TBBPA (log Kow=

8.02) is lower than that of caffeine (log Kow = -0.07) which is the opposite of the prediction. Clearly, other properties impact removal such as molecular size, structure, ionic versus neutral form, pH of the water, DOC concentration of water, and type of PAC.

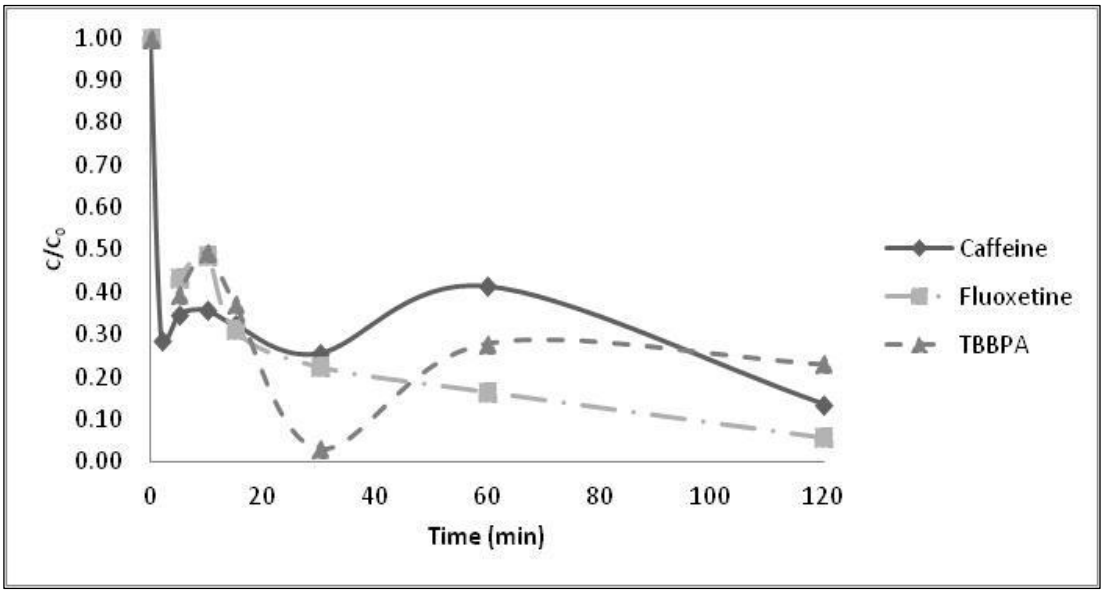


Figure 3.7: Removal of CEECs at PAC dose of 5mg/L over time.

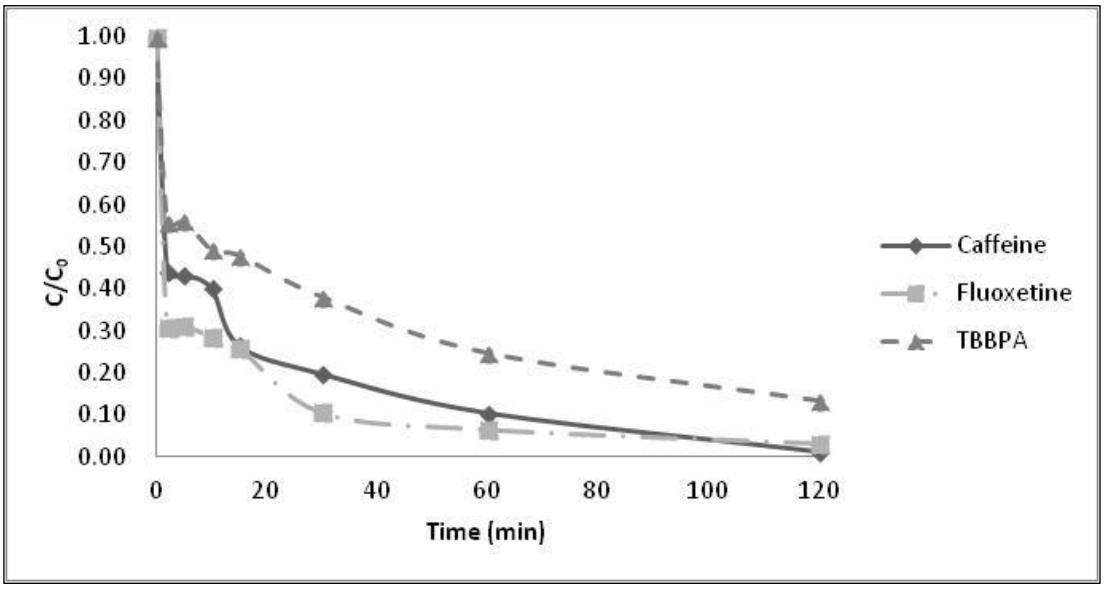


Figure 3.8: Removal of CEECs at PAC dose of 10mg/L over time.

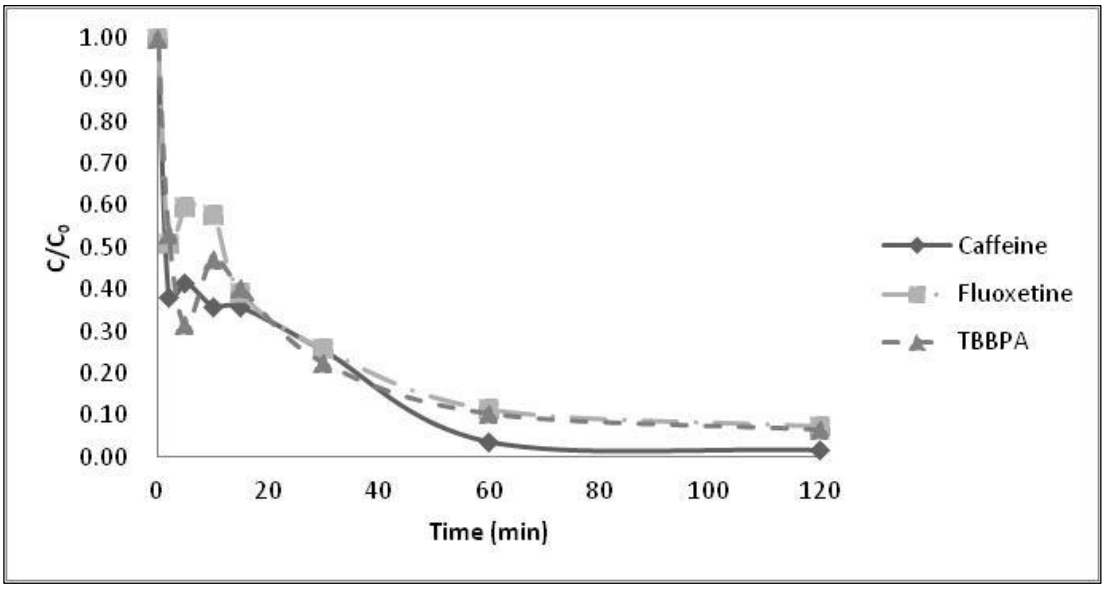


Figure 3.9: Removal of CEECs at PAC dose of 20mg/L over time.

Table 3.3: First order kinetic rate (M L^{-1} s^{-1}) of adsorption of CEECs by PAC.

PAC dose	Caffeine	Fluoxetine	TBBPA
5mg/L	0.0077	0.0176	0.0057
10mg/L	0.0297	0.0206	0.0125
20mg/L	0.0303	0.0187	0.0178

Knappe et al. (2012) evaluated PAC adsorption of pharmaceuticals at different pH values, PAC dose, water matrix, and type of PAC. They found that the pH at which neutral forms of pharmaceuticals prevailed was the optimum condition for their removal. The pH of the water in this current study was approximately 7, which, based on their pKa values (Table 1.2 of Section 1.3), indicates that caffeine, fluoxetine and TBBPA were in neutral form, and would have predicted removal which is supported by the results here. Knappe et al. (2012) further showed increased adsorption of pharmaceuticals at higher PAC doses, which is also the case in this study.

Knappe et al. (2012) also evaluated the effect of PAC addition during coagulation on pharmaceutical, DOC and turbidity removal. Their results indicated that while no significant increase in DOC and turbidity removal occurred compared to coagulation without PAC, there was a significant increase in removal of the pharmaceuticals. Taking into account the high removal of CEECs with PAC shown in this current study, its use during drinking water treatment would significantly reduce CEEC concentration before disinfection and reduce the formation of byproducts resulting from interaction with the disinfectant.

3.4 Chlorination and UV Treatment Byproducts

3.4.1 Chlorination Byproducts

Chlorine-treated CEECs quenched with ascorbic acid were directly infused onto the MS to scan for individual product ions and compared to those of the untreated sample as described in Section 2.2.5. Table 3.4 lists the parent and daughter ions if they were found for each CEEC after chlorination. A quenched chlorine solution was also infused but no ions were observed in the selected mass range. The reactivity of the CEECs is evaluated by the change in signal of the parent ion in the chlorine-treated sample. Fluoxetine, caffeine, and iohexol were not as reactive because the parent ions were still

very abundant in the treated solution compared to those in the untreated LGW solution (Table 3.4), whereas atorvastatin and tetracycline were very reactive. The reactivity can be explained by the substituent effect on electophilic aromatic substitution reactions. Atorvastatin and tetracycline contain activating substituents (-NR_2 and -OH), whereas fluoxetine and iohexol contain deactivating substituents (-CF_3 and -I). TBBPA was not detectable in this experiment. The lack of reactivity with caffeine is likely due to the highly substituted aromatic ring sterically hindering the ability of free chlorine to approach the molecule (Kaplan, 2011). A list of the parent and daughter ions of each CEEC in LGW is listed in Table 3.5.

Table 3.4: Ions found in the chlorine-treated CEEC solution (* indicates ions that are present in the untreated sample, ions in bold may contain chlorine)

CEEC (m/z, ion count, ESI mode of parent ion before treatment)	**Ionization**	**Parent ion (approximate ion count)**	**Daughter ion 1 m/z (ion count)**	**Daughter ion 2 m/z (ion count)**
Atorvastatin (581.4, 1E07, (+)ESI)	+ESI	**80.9 (9E06)**	**ND**	**ND**
		82.9 (3E06)	**ND**	**ND**
		215.0 (4E07)	ND	ND
		247.1 (1E07)	229.0 (5.4E05)	167.0 (6.4E04)
		407.0 (7E06)	214.0 (1.0E06)	ND
		476.4 (2E06)	ND	ND
		597.3 (3E07)	**462.3 (1.3E6)**	**554.3 (3.3E05)**
		598.3 (1E07)	554.6 (1.7E05)	462.7 (9.0E04)
		599.3 (3E06)	**556.3 (3.1E04)**	**ND**
		613.2 (4E06)	ND	ND
		631.2 (1E07)	**308.4 (8.3E04)**	**458.3 (1.5E04)**
		632.2 (3E06)	237.3 (1.7E04)	124.3 (2.5E03)
		633.2 (3E06)	**ND**	**ND**
		634.2 (3E06)	ND	ND
	-ESI	60.8 (4E07)	ND	ND
		76.8 (7E07)	ND	ND

ND=Not detected

Table 3.4 (continued): Ions found in the chlorine-treated CEEC solution (* indicates ions that are present in the untreated sample, ions in bold may contain chlorine)

CEEC (m/z, ion count, ESI mode of parent ion before treatment)	Ionization	Parent ion (approximate ion count)	Daughter ion 1 m/z (ion count)	Daughter ion 2 m/z (ion count)
Fluoxetine (310.0, 4E07, (+)ESI)	+ESI	62.7 (8E06)	ND	ND
		80.8 (1E07)	**79.7 (2.8E05)**	**ND**
		82.8 (3E06)	**ND**	**ND**
		90.0 (1E07)	88.9 (7.1E05)	72.6 (1.1E04)
		148.2 (2E07)	102.9 (3.7E05)	69.0 (1.1E05)
		215.0 (1E08)	ND	ND
		*310.0 (1E08)	ND	ND
		*360.2 (2E07)	147.9 (8.8E05)	163.9 (7.4E05)
	-ESI	173.0 (1E07)	120.4 (1.7E03)	75.4 (1.6E03)
		175.2 (3E06)	ND	ND
		177.0 (1.5E06)	ND	ND
		193.2 (1E07)	ND	ND
		206.0 (4E07)	ND	ND
		208.4 (4E07)	ND	ND
		209.4 (4E07)	**64.2 (2.2E03)**	**177.7 (1.2E03)**
		210.1 (2E07)	ND	ND
		211.1 (1E07)	**ND**	**ND**
		219.4 (5E07)	ND	ND
		220.0 (2.5E07)	ND	ND
		341.1 (5E06)	88.6 (6.4E03)	ND
Caffeine-d3 (198.1, 6E07, (+)ESI)	+ESI	80.9 (1E07)	ND	ND
		86.0 (1E07)	68.7 (1.2E04)	ND
		112.9 (2E07)	71.9 (2.0E04)	56.5 (1.1E05)
		141.0 (2E07)	94.7 (9.4E05)	83.1 (5.5E05)
		157.0 (1E07)	110.8 (3.7E05)	ND
		171.0 (1E07)	152.3 (7.6E04)	85.8 (2.9E04)
		175.0 (2E07)	62.9 (8.8E05))	130.8 (1.4E06)
		*198.1 (6E07)	ND	ND
		215.0 (1E08)	ND	ND
	-ESI	*60.8 (7E07)	ND	ND
		*61.9 (7E07)	ND	ND
		*76.8 (7E07)	ND	ND
		173.0 (5E06)	ND	ND
		219.1 (4E06)	98.8 (1.5E04)	128.6 (1.1E04)

ND=Not detected

Table 3.4 (continued): Ions found in the chlorine-treated CEEC solution (* indicates ions that are present in the untreated sample, ions in bold may contain chlorine)

CEEC (m/z, ion count, ESI mode of parent ion before treatment)	Ionization	Parent ion (approximate ion count)	Daughter ion 1 m/z (ion count)	Daughter ion 2 m/z (ion count)
Iohexol (843.9, 3E06, (+)ESI)	+ESI	*65.0 (1E07)	ND	ND
		157.0 (5E06)	ND	ND
		175.0 (5E06)	130.8 (4.5E05)	62.8 (3.3E05)
		197.0 (4E06)	62.8 (4.3E05)	153.0 (2.6E05)
		*198.1 (3E06)	ND	ND
		199.0 (1E07)	62.7 (9.7E04)	56.8(3.6E03)
		215.0 (7E07)	ND	ND
		247.0 (2E07)	ND	ND
		255.1 (6E06)	156.9 (8.8E05)	144.8 (4.8E05)
		407.0 (1E07)	214.9 (1.7E07)	ND
		*843.9 (8E06)	ND	ND
	-ESI	*60.9 (4E07)	ND	ND
		*76.8 (4E07)	ND	ND
Tetracycline (445.3, 1E07, (+)ESI)	+ESI	**80.8 (2E07)**	**ND**	**ND**
		82.9 (6E06)	**ND**	**ND**
	-ESI	*60.8 (5E07)	ND	ND
		61.8 (5E07)	ND	ND
		*76.7 (5E07)	ND	ND

ND=Not detected

Table 3.5: Ions found in CEEC solution in LGW

Compound	Ionization	Parent ion (approximate ion count)
Atorvastatin	+ESI	559.8 (1E07)
		575.5 (5E06)
		581.2 (1E07)
	-ESI	557.0 (1E08)
Fluoxetine	+ESI	149.0 (1E07)
		279.1 (1E06)
		310.2 (4E07)
		360.2 (5E06)
Caffeine-d3	+ESI	198.1 (6E07)
		220.1 (1E07)
	-ESI	60.8 (2E07)
		65.8 (2E07)
		76.8 (2E07)
Iohexol	+ESI	65.0 (2E07)
		198.1 (1E07)
		822.9 (1E06)
		843.9 (3E06)
	-ESI	60.8 (3E07
		76.8 (6E07
Tetracycline	+ESI	158.1 (1E07)
		445.1 (1E07)
	-ESI	59.9 (1E08)
		60.9 (1E08)
		76.8 (1E08)

Chlorine Isotopes

In order to identify whether the byproducts contain chlorine, the isotope ratios between ^{35}Cl, ^{37}Cl, and ^{39}Cl were investigated (Table 3.6). The ions listed in bold in Table 3.4 are those that exhibit the correct isotope ratio for chlorine incorporation and in all cases suggest only a single atom of chlorine incorporation. For example, the ions with m/z 80.9/82.9 in the ratio 3:1 are present in atorvastatin, fluoxetine and tetracycline

and could be indicative of chloroethanol, a toxic, colorless liquid. The relative proportions of these ions to the parent ion are much lower for fluoxetine (which contains no -OH groups) than for tetracycline and atorvastatin both of which contain multiple -OH groups giving this observation some credibility.

Table 3.6: Relative intensities (highest ion count set to 100%) of isotopes for up to 3 chlorine atoms in an ion (Glagovich 2007)

	Cl	**2Cl**	**3Cl**
$(M)^+$	100	100	100
$(M+2)^+$	31	65	95
$(M+4)^+$		10	31

Structure of Byproducts

Some possible structures based on the mass to charge of ionizable chlorination byproducts are shown in Figures 3.10-3.13. The nitrogen rule (even number of nitrogen atoms in an ion = even mass, odd number of nitrogen atoms in an ion = odd mass) was used to account for the number of nitrogens in the structure. For byproducts that contain daughter ions, some were also hypothesized (see Appendix B, Table B-1) based on Glagovich (2007). The byproducts confirm the mechanism of electrophilic attack by chlorine of the aromatic rings, especially in the case of iohexol which has only one aromatic ring that appears to be completely broken apart. Although the identity of chlorine-incorporated byproducts could not be reasonably conjectured, their identification would be further confirmation that electrophilic substitution had occurred.

The structure of the byproducts can be indicative of bioactivity so that if a major part of the parent structure is retained after reaction so could its bioactivity. Compared to the parent structure of atorvastatin (Figure 3.10), the byproducts at m/z 215.0 and 247.1 do not have much of the original structure intact and the biological activity of these byproducts will probably have changed. This is also the case for all the byproducts hypothesized for fluoxetine, caffeine and iohexol. The byproduct at m/z 407.0 from atorvastatin, on the other hand, still contains much of the parent structure and might still possess some biological activity. The loss of parent structure and bioactivity does not necessarily suggest that the byproduct is less toxic than the parent compound. Other studies have shown that the byproducts of chlorination have higher toxicity than the parent compound (Bedner & MacCrehan 2006b; Duirk et al. 2011; Richardson et al. 2008).

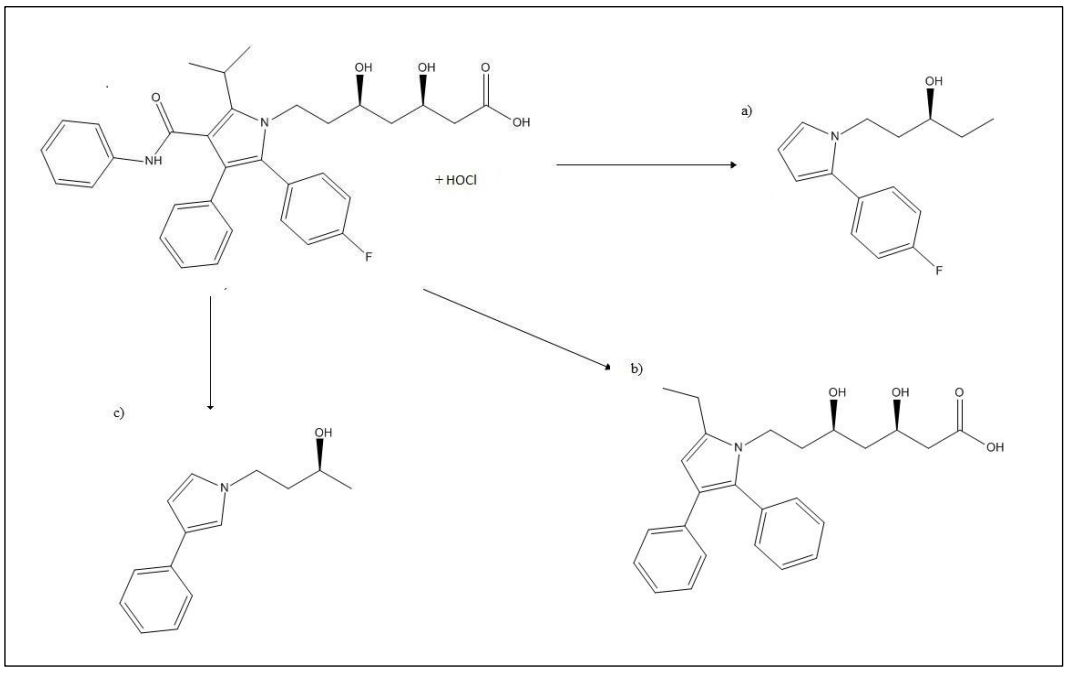


Figure 3.10: Possible structures of the chlorination byproducts of atorvastatin at m/z, a)247.1, b)407.0 and c)215.0.

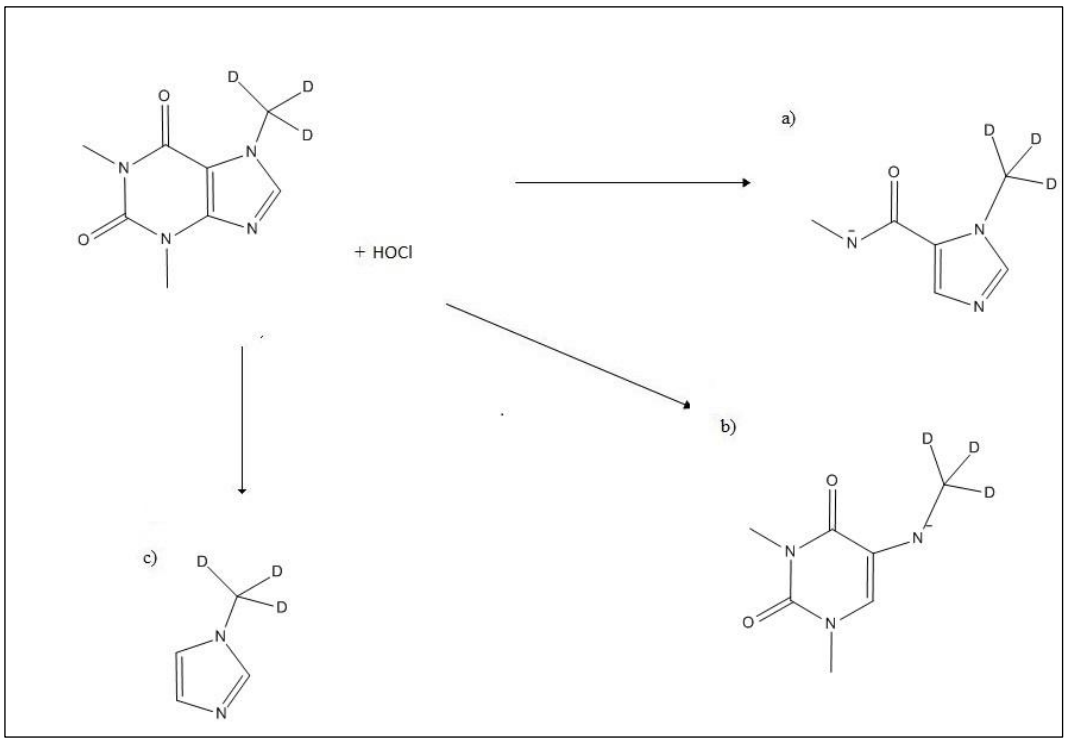


Figure 3.11: Possible structures of the chlorination byproducts of caffeine at m/z, a)141.0, b)171.0 and c)86.0.

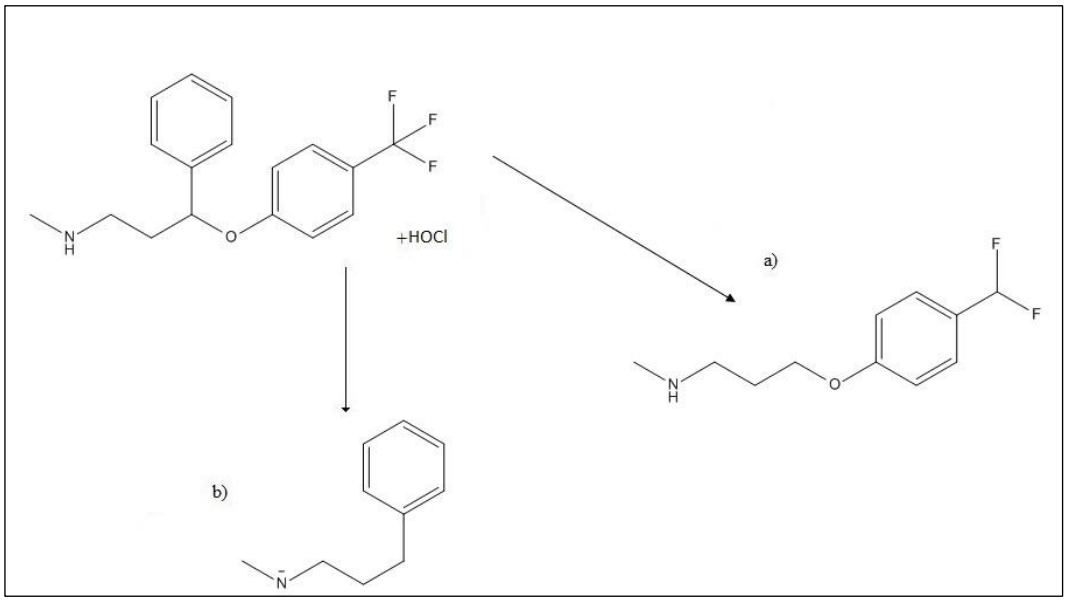


Figure 3.12: Possible structures of the chlorination byproducts of fluoxetine at m/z, a)215.0 and b)148.2.

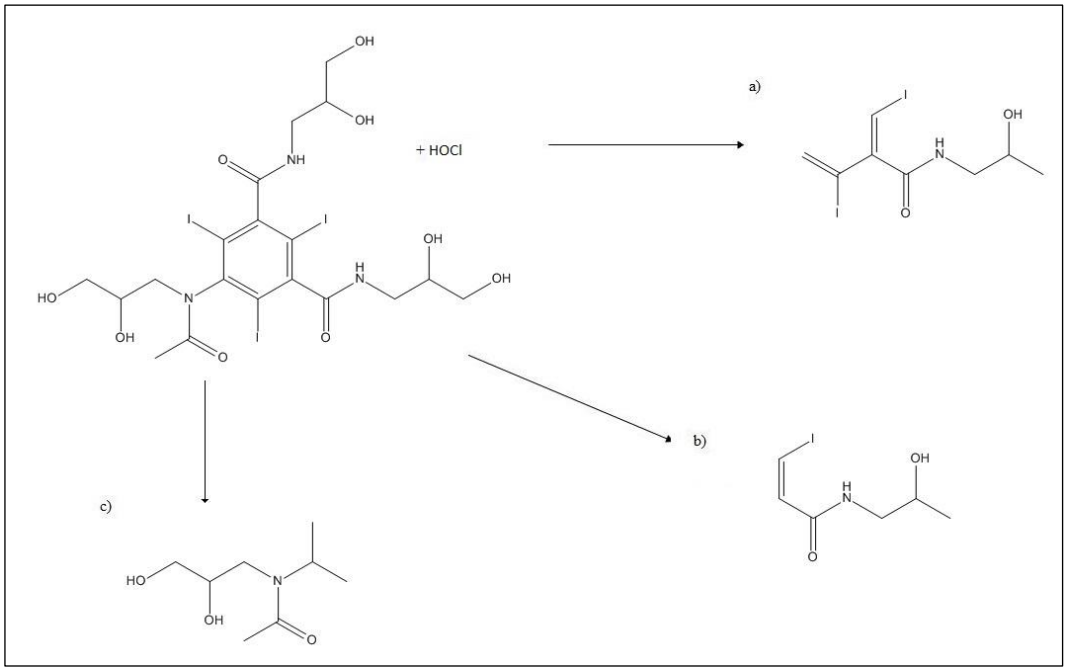


Figure 3.13: Possible structures of the chlorination byproducts of iohexol at m/z, a) 407.0, b)255.1, and c)175.0.

3.4.2 UV Byproducts

A list of the parent and daughter ion masses found for each CEEC after UV treatment according to the experiments described in Section 2.2.5 is presented in Table 3.7. Atorvastatin and iohexol were very reactive under UV irradiation, whereas fluoxetine, and in particular caffeine and tetracycline, were not. The degradation of chemicals under UV irradiation can occur by direct or indirect photolysis (Section 1.6.3). Direct photolysis breaks down the chemical through absorption of the incident wavelengths which are in the range 200-400nm in the case of MP-UV. The structures that usually absorb in this range contain delocalized π-electron systems which are found in aromatic rings and conjugated double bonds. Chemicals containing these structures

undergo excitation when irradiated with UV and can either experience structural change or energy loss from heat or light emission indicating that there is no simple rule to predict reactivity based on chemical structure (Schwarzenbach et al. 2003). Indirect photolysis occurs through the generation of radicals that are extremely reactive but non selective with most chemicals. Hence, the structures of CEECs cannot definitively predict their reaction during UV treatment. The number of byproducts from UV treatment with the targeted CEECs is much lower than those identified from chlorination.

Table 3.7: Ions found in UV-treated sample CEEC solution (* ions that are present in CEEC standard)

CEEC (m/z, ion count, ESI mode of parent ion before treatment)	Ionization	Parent ion (approximate ion count)	Daughter ion 1 m/z (ion count)	Daughter ion 2 m/z (ion count)
Atorvastatin (581.4, 1E07, (+)ESI)	+ESI	56.9 (1E07)	ND	ND
		438.4 (1E06)	ND	ND
		513.2 (1E06)	120.0 (3.8E4)	395.1 (2.5E03)
		*559.3 (5E05)	ND	ND
		595.4 (2E06)	ND	ND
	-ESI	60.9 (5E07)	ND	ND
		65.9 (5E07)	ND	ND
		76.7 (2.5E07)	ND	ND
Fluoxetine (310.0, 4E07, (+)ESI)	+ESI	90.9 (3E07)	ND	ND
		115.0 (1E07)	88.9 (1.4E07)	ND
		117.0 (2E07)	115.0 (6.6E06)	ND
		*149.1 (1E07)	117.8 (2.3E6)	115.9 (5.6E5)
		164.1 (1E07)	109.1 (6.3E4)	76.8 (6.0E04)
		166.0 (2E07)	104.9 (1.7E05)	119.2 (4.2E04)
		259.1 (9E06)	230.8 (4.6E05)	90.8 (3.3E05)
		*310.2 (2E07)	ND	ND
	-ESI	60.8 (7E06)	ND	ND
		76.8 (7E06)	ND	ND
		91.8 (7E06)	ND	ND
Caffeine-d3 (198.1, 6E07, (+)ESI)	+ESI	86.0 (2E07)	ND	ND
		113.0 (2E07)	85.8 (6.4E05)	57.5 (4.3E04)
		143.0 (3E07)	58.1 (1.7E06)	97.1 (2.2E05)
		*198.1 (8E07)	ND	ND
		*220.1 (3E06)	ND	ND
	-ESI	*60.8 (1E07)	ND	ND
		*65.8 (1E07)	ND	ND
		*76.8 (1E07)	ND	ND
		196.1 (4.1E06)	98.7 (3.9E03)	152.5 (4.6E03)
		229.4 (7E06)	210.5 (2.4E04)	139.5 (6.1E03)

ND= Not detectable

Table 3.7 (continued): Ions found in UV-treated sample CEEC solution (* ions that are present in CEEC standard)

CEEC (m/z, ion count, ESI mode of parent ion before treatment)	Ionization	Parent ion (approximate ion count)	Daughter ion 1 m/z (ion count)	Daughter ion 2 m/z (ion count)
Iohexol (843.9, 3E06, (+)ESI)	+ESI	*65.0 (1E07)	ND	ND
		91.9 (5E06)	ND	ND
		149.0 (4E06)	103.1 (4.7E05)	84.8 (1.8E05)
		*198.1 (6E06)	ND	ND
	-ESI	*60.9 (5E07)	ND	ND
		65.9 (5E07)	ND	ND
		*76.8 (5E07)	ND	ND
Tetracycline (445.3, 1E07, (+)ESI)	+ESI	*158.0 (1E07)	ND	ND
		*445.1 (1E07)	ND	ND
	-ESI	*59.9 (1E08)	ND	ND
		*60.9 (1E08)	ND	ND
		*76.8 (1E08)	ND	ND

ND=Not detectable

Table 3.8 lists the calculated molar absorptivity at the wavelength of maximum absorption of targeted CEECs in the UV range for 10mg/L solutions. The chemicals with higher molar absorptivity (e.g. iohexol and atorvastatin) seem to correspond with those of highest reactivity in the UV irradiation experiments. However, tetracycline has a high molar absorptivity at 274nm but is not reactive with MP UV irradiation, suggesting molar absorptivity is not the sole predictor of reactivity. Figure 3.14 shows the output of the UV MP lamp illustrating a broad spectrum of irradiation.

Table 3.8: Molar absorptivity of CEECs (10mg/L) at their λ_{max} .

CEEC	λ_{max} (nm)	Molar absorptivity (M^{-1} cm^{-1})
Atorvastatin	241	17763
Caffeine	272	10060
	205	27596
Fluoxetine	226	11599
Iohexol	245	33583
TBBPA	206	6146
Tetracycline	274	16176
	362	13732

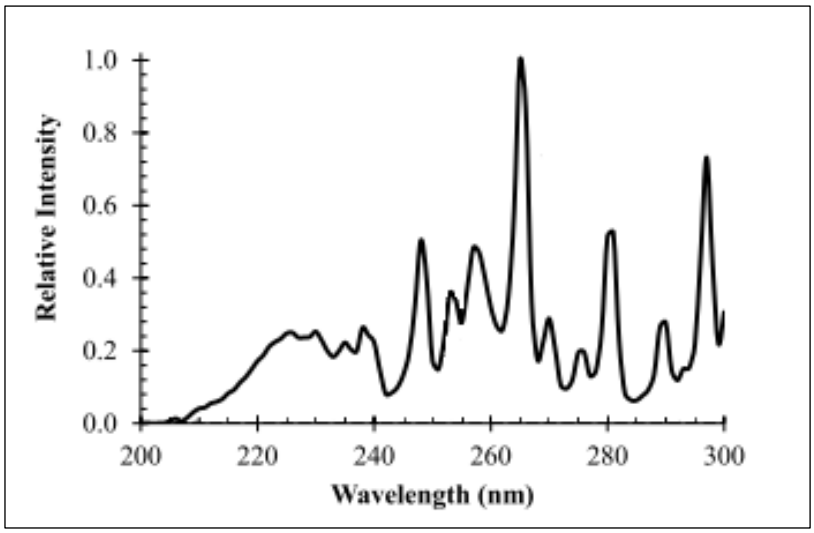


Figure 3.14: Spectrum of lamp output for MP UV.

Structure of Byproducts

The hypothesized UV byproduct structures are shown in Figures 3.15-3.17 while those with daughter ions are described in Appendix B, Table B-2 based on Glagovich (2007). CEECs whose byproducts have the highest masses are most likely to retain much of the original structure and perhaps bioactivity. However, UV disinfection is usually not used alone in full-scale treatment and is paired with a chemical disinfectant such as chlorine or chloramines in drinking water treatment. The UV treatment can

break down the original CEEC into a smaller molecule which may or may not be reactive with chlorine. Lyon (2012) observed an increase in DBP formation from adding chlorine after UV treatment of surface water at high UV doses but not at lower doses. However, only the known DBPs were analyzed; therefore, it cannot be certain that low UV doses do not generate DBP precursors.

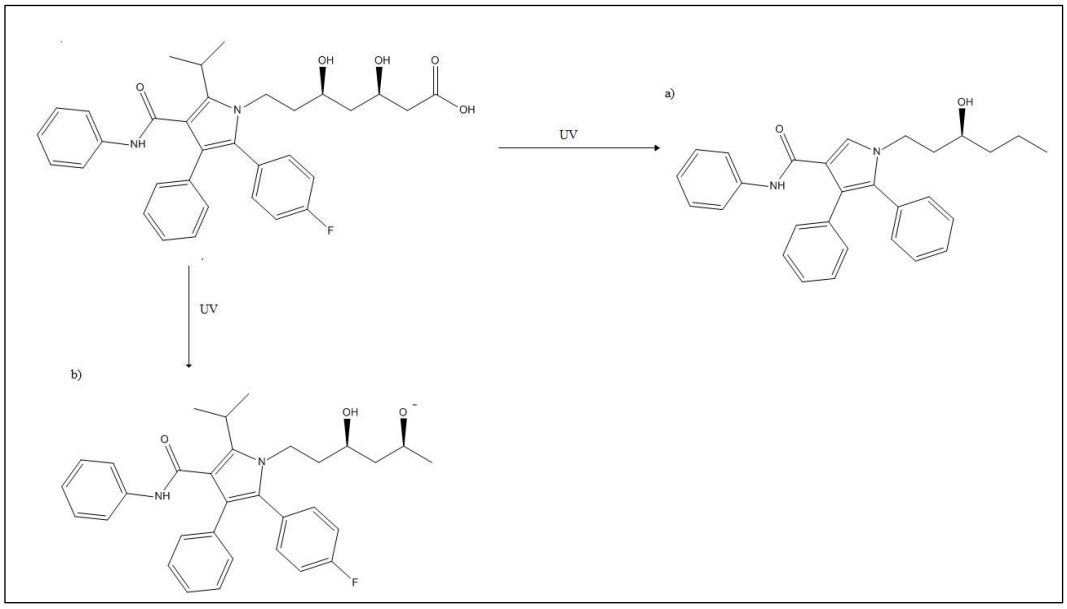


Figure 3.15: Hypothesized structures of UV byproducts of atorvastatin at m/z, a) 438.4 and b) 513.2.

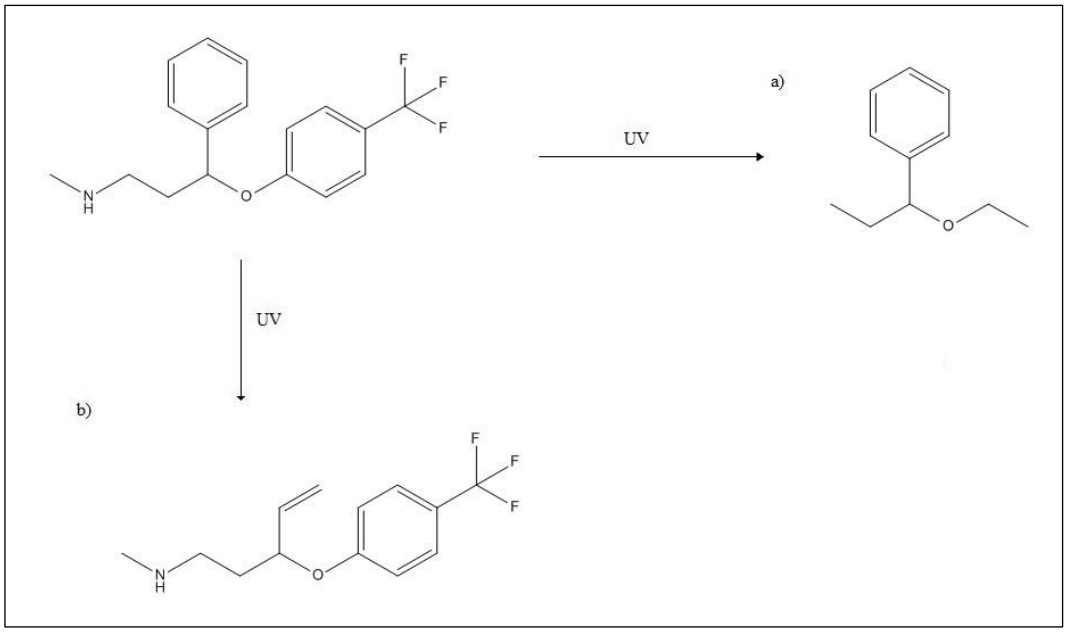


Figure 3.16: Hypothesized structures of UV byproducts of fluoxetine at m/z, a) 164.1 and b) 259.1.

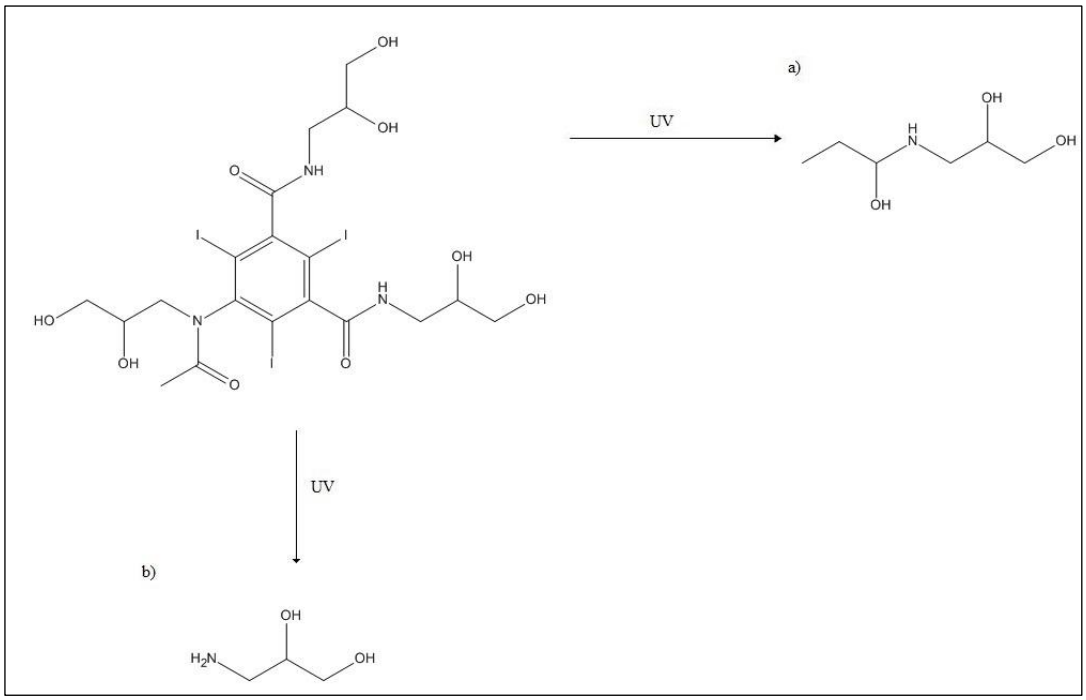


Figure 3.17: Hypothesized structures of UV byproducts of iohexol at m/z, a) 149.0 and b) 91.9.

If chlorine was used as the chemical disinfectant following UV treatment, the UV byproducts containing aromatic structures would probably react, forming chlorinated byproducts through electrophilic substitution. This is especially the case with atorvastatin which appears to retain most of its parent structure. The fluoxetine byproduct at m/z 259.1 appears to have retained the deactivating $–CF_3$ substituent, rendering it less reactive to chlorine. On the other hand, the byproduct at m/z 166.0 did not retain the deactivating substituent and may actually have a weakly activating substituent, suggesting it might be more active than fluoxetine towards chlorine.

3.5 Tetracycline Case Study

In a separate experiment 1mg/L tetracycline in reaction with 20mg/L as Cl_2 free chlorine and chloramine was studied in more detail according to Section 2.2.7. Reactivity of tetracycline with both disinfectants was high as reflected in the absence of the original chemical in the treated sample and a large amount of halogenated byproduct measured by TOX. Since reaction with chlorine produces the highest level of TOX its reaction was studied in more depth.

A solution of tetracycline in LGW and chlorinated tetracycline quenched in LGW were analyzed for their UV absorbance spectrum as described in Section 2.2.6. Tetracycline has a λ_{max} at 276nm and 356nm (Figure 3.18). If the reaction with chlorine

resulted in the incorporation of chlorine on the benzene rings of tetracycline, the λ_{max} should have increased (Robinson et al. 2005). The quenched chlorinated solution has neither of the original peaks but has a λ_{max} at 232nm (Figure 3.19) suggesting that the ring structure of the tetracycline may no longer be intact. Further characterization of the sample used direct infusion MS but no product was identified. In order to identify lower molecular weight and perhaps more volatile byproducts another more concentrated solution of chlorinated tetracycline was analyzed using GC-ECD after liquid-liquid extraction as described in Section 2.2.6 and a product peak was found. In order to identify this product peak (See Appendix B, Figure B-1), the extract was analyzed using GC-MS and the mass spectrum of that peak shown in Figure 3.20 is a match for chloroform which has previously been identified as a product of oxytetracycline (Xu et al. 2012). Since the two chemicals have similar structures they might be expected to have similar byproducts. However, Xu et al. (2012) identified additional reaction products of chlorine with oxytetracycline including dichloroacetone, trichloroacetone, dichloroacetonitrile, trichloroacetonitrile and trichloronitromethane but these were not seen in the chlorination byproducts of tetracycline. Xu et al. (2012) used 9.2mg/L of tetracycline to react with 26.3mg/L of chlorine under varying pH conditions controlled by buffer solutions. Buffer solution was not used in this current study to prevent other reactions with chlorine. Additionally, the concentrations of both tetracycline and chlorine in this current study are considerably higher than those in Xu et al. (2012). Nonetheless, chloroform is one of the regulated DBPs in drinking water.

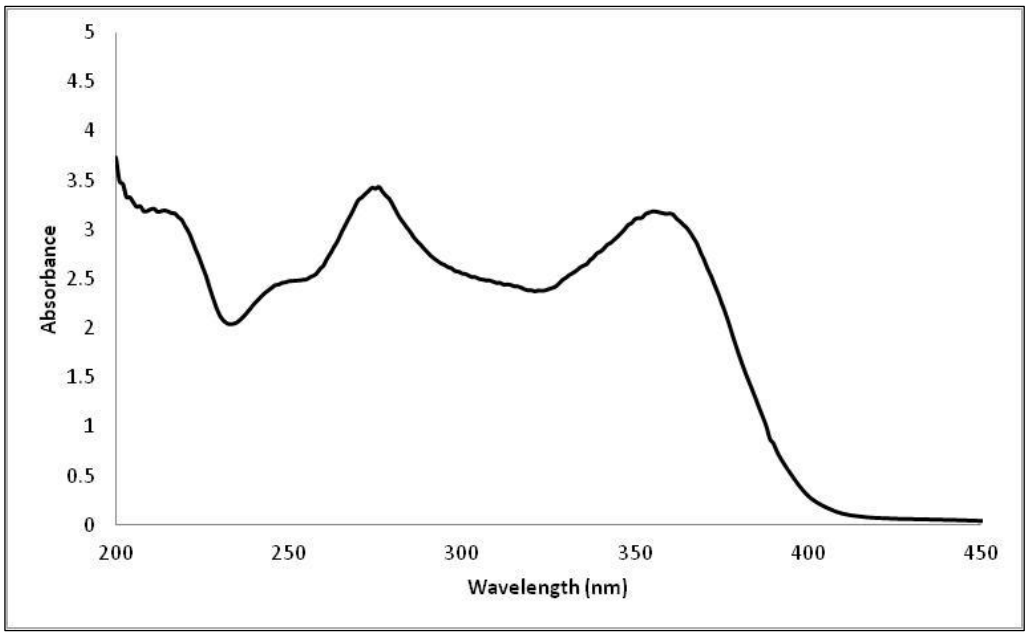


Figure 3.18: Absorbance spectrum of 10mg/L tetracycline in LGW, with λ_{max} at 276nm and 356nm.

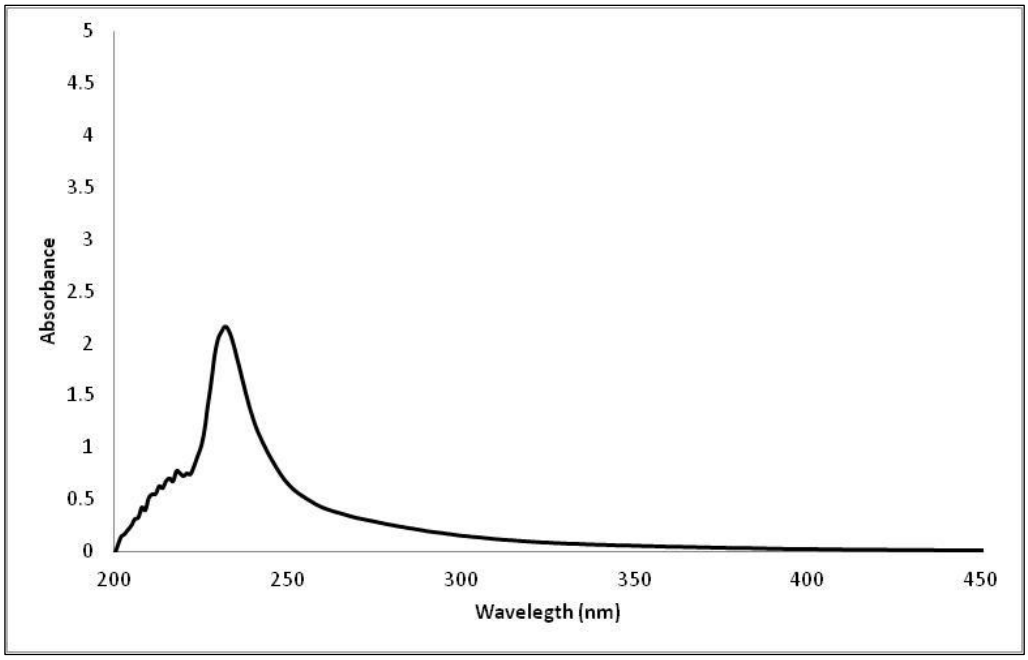


Figure 3.19: Absorbance spectrum of chlorinated tetracycline (10mg/L tetracycline, 31.9mg/L chlorine), with λ_{max} at 232nm.

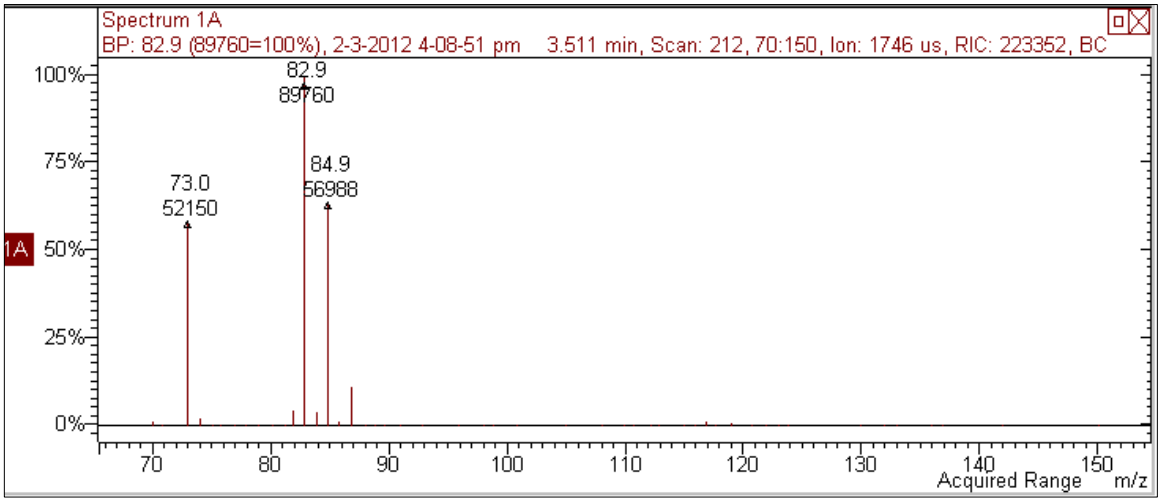


Figure 3.20: Spectrum of product peak (chloroform) from GC-MS analysis of chlorinated tetracycline.

The kinetics of chloroform and TOX formation from chlorination of tetracycline were evaluated at pH 7 and 9 as described in Section 2.2.6. Figure 3.21 shows the distribution of HOCl and OCl^- as a function of pH. At pH 7 HOCl predominates while at pH 9 OCl^- is the dominant species. HOCl and OCl^- both react though the same mechanism of aromatic substitution and oxidation; however, HOCl is the more reactive species because it is a stronger oxidant. OCl^- replenishes HOCl when the latter is consumed to reestablish equilibrium in the system. Therefore, when HOCl is more abundant the reaction with tetracycline produces more chloroform. An evaluation of the concentration of chloroform formed over time at different pH values shows that at each timed point it is higher at pH 7 than at pH 9 (Figure 3.22). This is also the case for the formation of TOX, shown in Figure 3.23, but the impact of pH is larger.

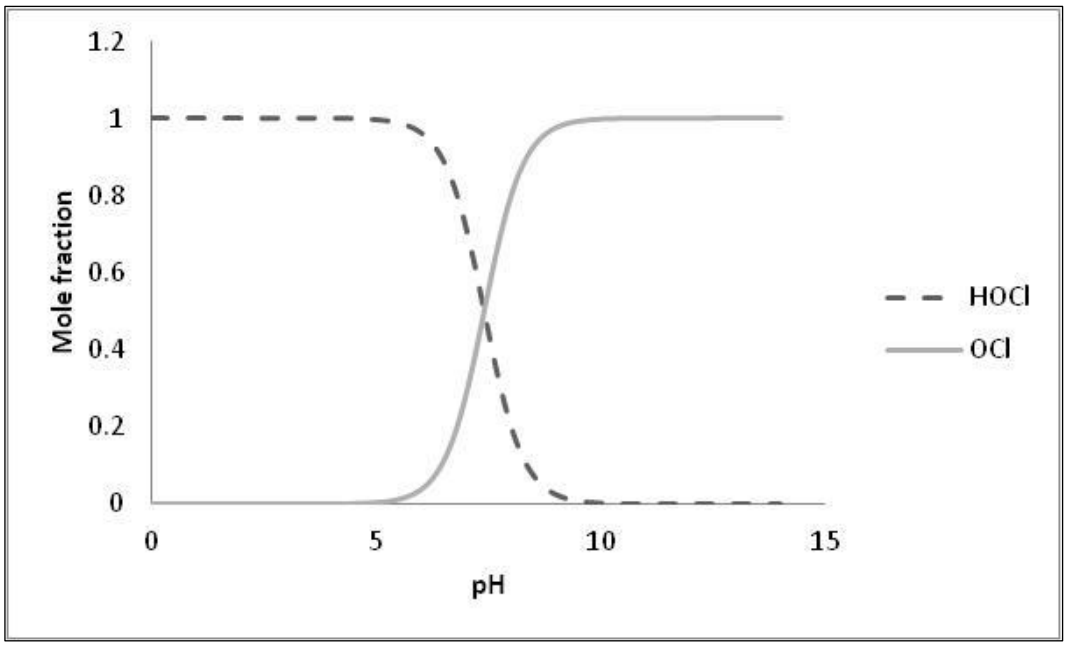


Figure 3.21: Distribution of HOCl and OCl^- with varying pH.

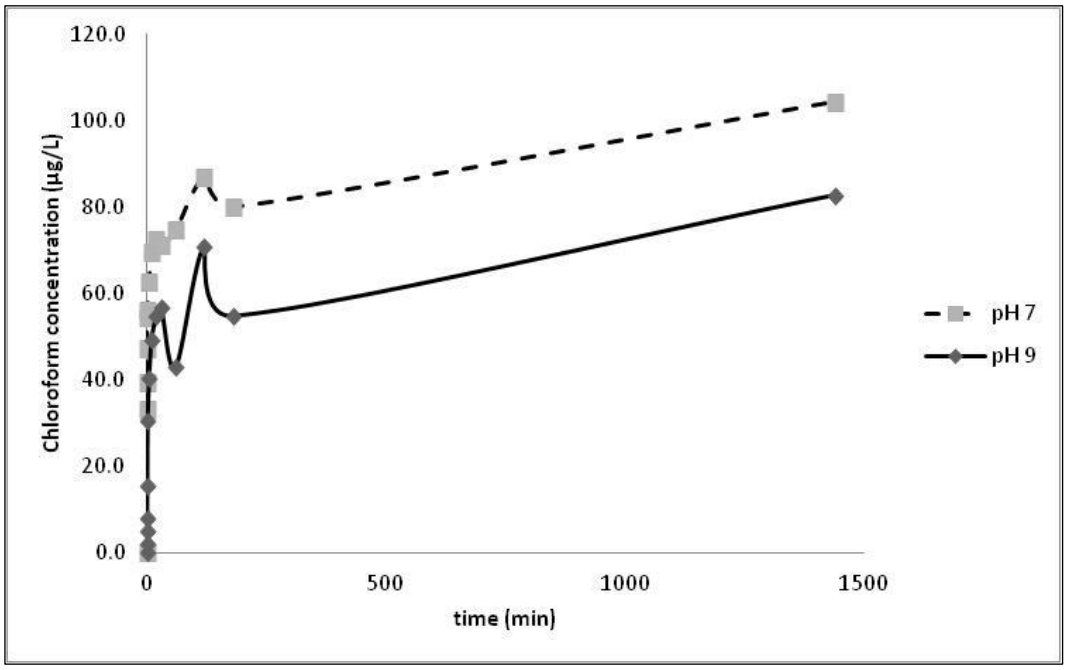


Figure 3.22: Comparison of the formation of chloroform over time by chlorination of tetracycline with respect to pH.

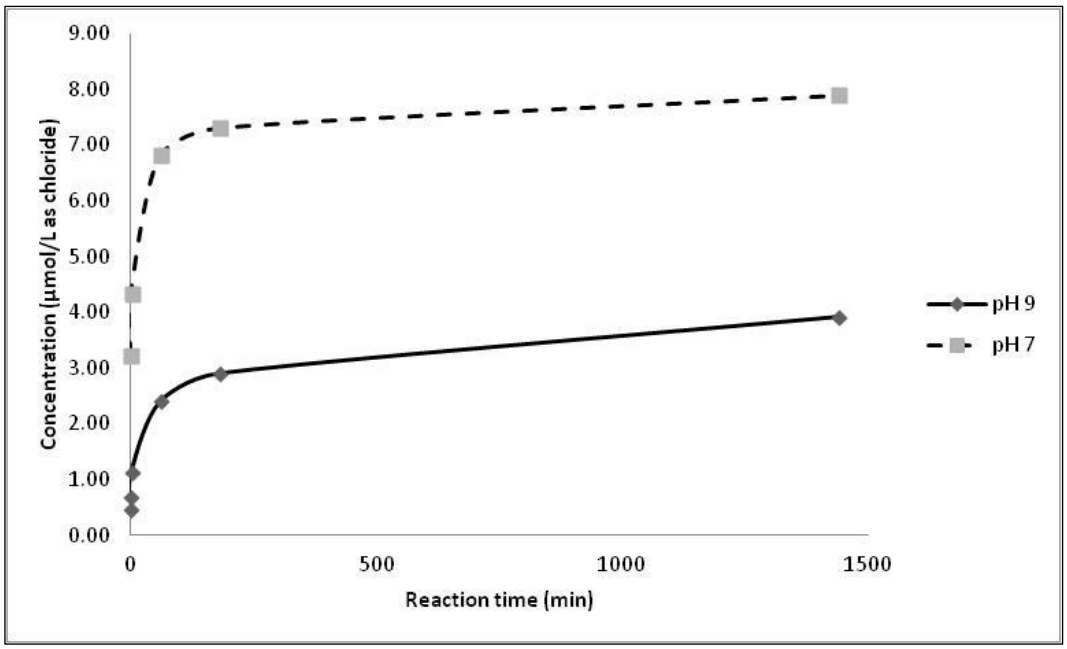


Figure 3.23: Comparison of the formation of TOX by chlorination of tetracycline over time with respect to pH.

Figures 3.24 and 3.25 show the concentration of chloroform and TOX formed in µmol/L of chlorine at pH 7 and pH 9, respectively. At pH 7, 27% of the TOX formed was accounted for by chloroform whereas at pH 9 this increased to 50%.

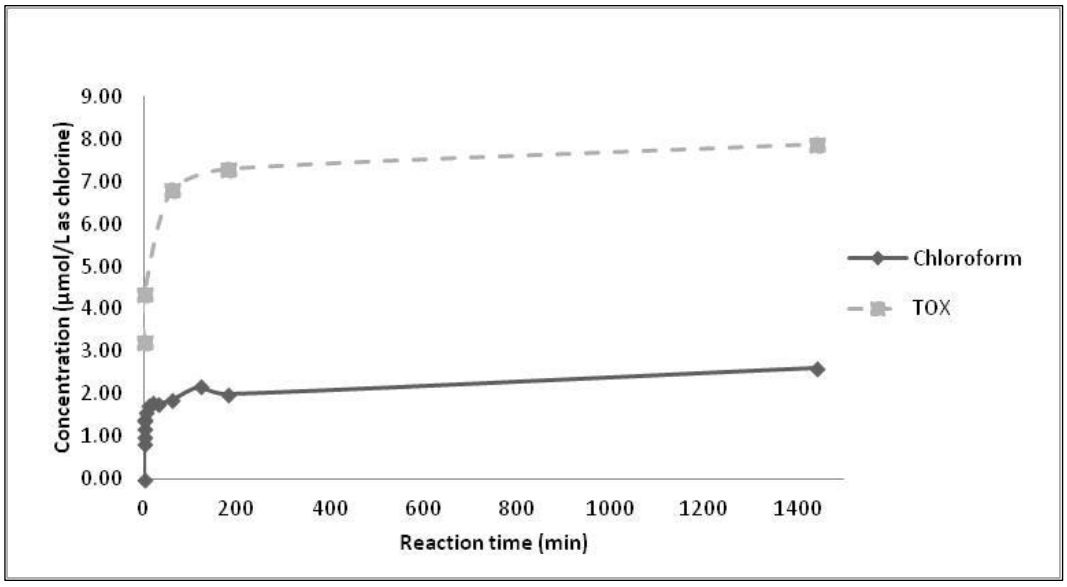


Figure 3.24: Comparison of TOX and chloroform formation from chlorination of tetracycline at pH 7.

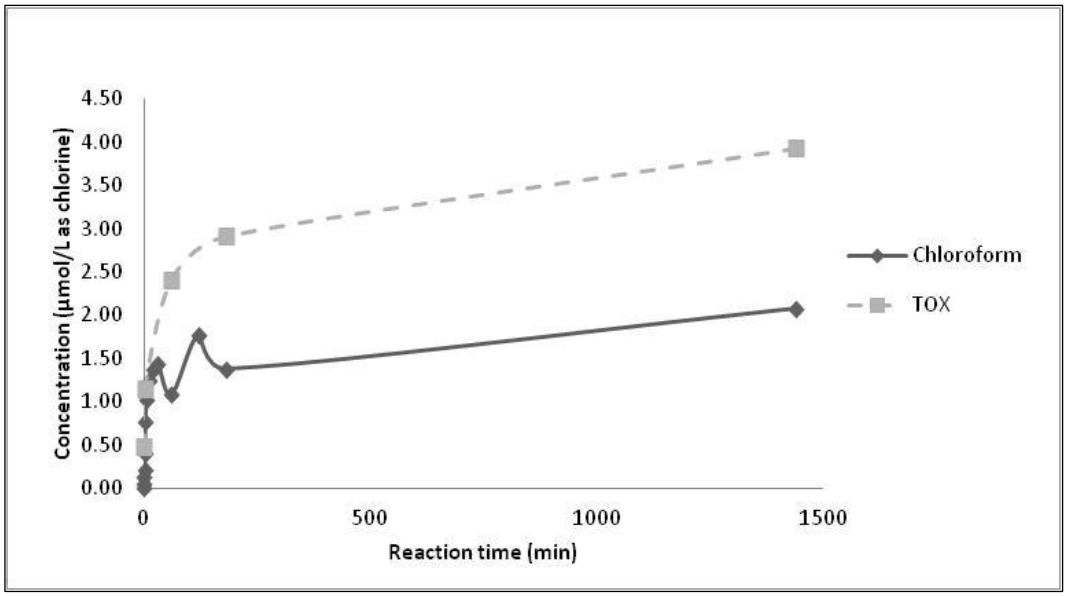


Figure 3.25: Comparison of TOX and chloroform formation from chlorination of tetracycline at pH 9

The rate of formation of chloroform and TOX could not be calculated from kinetic rate equations but linear regressions fitted through different time segments during the reaction were used to calculate formation rates at different times (Table 3.9-3.10).

Comparing the initial rates of formation, for TOX it is greater than the rate of chloroform formation; however, this difference is smaller at pH 7 compared to pH 9. Also noting the difference between rate of formation of chloroform between pH 7 and pH 9, the initial rates are very different but then become about the same. This suggests the initial concentration of HOCl available for reaction is the main determinant in how much chloroform forms during chlorination of tetracycline.

The rate of formation of chloroform and TOX decreases rapidly over time. There were only two segments for TOX formation because TOX was only analyzed for a subset of the times of those analyzed for chloroform. Comparing the rates for segment 1, TOX formation was much faster than chloroform and formation was faster at pH 7 compared to pH 9. The formation of chloroform could be occurring through the reaction of chlorine with the -OH containing ring in tetracycline. In Gallard & Von Gunten (2002), the formation of THMs from reaction of chlorine with phenol was studied, but no reaction mechanism was suggested.

Table 3.9: Rate constants ($M\,sec^{-1}$) for the formation of chloroform from chlorination of tetracycline at pH 7 and 9.

	Time	**Rate constant at pH 7**	**Rate constant at pH 9**
Segment 1	0 min - 1min	19300	6600
Segment 2	2 min - 20 min	400	500
Segment 3	30 min -24hours	8	8

Table 3.10: Rate constants ($M\ sec^{-1}$) for the formation of TOX from chlorination of tetracycline at pH 7 and 9.

	Time	**Rate constant at pH 7**	**Rate constant at pH 9**
Segment 1	0 min - 1min	37200	11100
Segment 2	1 hour - 24 hour	10	20

In drinking water treatment, if tetracycline was present in the source water, it might not be removed through coagulation, depending on the DOC concentration in the source water (Section 3.2). PAC addition can remove tetracycline but if it is not used then tetracycline will be present during final disinfection. The use of UV pre-treatment, especially under typical operating doses will not likely breakdown tetracycline (see Section 3.4.2), which means that if it is present in source water it would likely generate some halogenated byproducts.

4. CONCLUSIONS

The effectiveness of bench scale drinking water treatment processes, and in particular disinfection with chlorine and UV on the removal of CEECs in surface water was evaluated in this thesis. This addresses the concern of whether current drinking water treatment processes can protect finished water from CEEC contamination if such chemicals are present in the source water. It is not possible to evaluate every one of the many CEECs in production and use so representative ones in high use found in many surface waters and having different structural properties were selected for this study. They were atorvastatin (FOC, lipid regulator), caffeine (stimulant), fluoxetine (FOC, antidepressant), iohexol (x-ray contrast agent), tetracycline (antibiotic) and TBBPA (flame retardant). The treatment processes evaluated were coagulation with aluminum and ferric sulfate, PAC, chlorination and MP-UV and elevated levels of CEECs were used compared to environmental concentrations so as to monitor trends with existing analytical methods. SPE was used to isolate the analytes from aquatic matrix, which was tested to have recoveries ranging from 64-154%.

Coagulation was not particularly effective in removing CEECs as predicted due to the high solubility of the selected chemicals. PAC on the other hand was

demonstrated to be highly effective, around 90% at the highest PAC dose (20mg/L), in the removal of three of the CEECs, fluoxetine, caffeine and TBBPA. Chlorination and UV irradiation were shown to transform the CEECs into byproducts to varying degrees. Atorvastatin and tetracycline were the most reactive to chlorine, while atorvastatin and iohexol were the most reactive under UV. Bioactivity was not measured in this study, but if the structures of the byproduct contain the moieties of the parent compound that were responsible for its activity then bioactivity may still be present in the treated water.

Although this study identified only a few of the byproducts from chlorination and UV treatment, albeit under conditions not representative of full-scale treatment, the results serve to show that residual CEECs in drinking water sources cannot be ignored. While coagulation was not shown to be a reliable and catch-all good barrier, PAC did remove a high percentage of all the CEECs evaluated in this study. If UV were used as a pretreatment to break down some of the more recalcitrant chemicals, overall removal could be improved. This study did show that UV can change a relatively inert chemical into one that is more reactive with chlorine, and so if activated carbon was not used between or before the two treatments, new chlorination byproducts of unknown toxicity might persist into consumers' drinking water. Since the effects of chronic low level human exposure to such chemicals is unknown, it is prudent to provide a dual strategy of watershed protection with adjustments to drinking water treatment to ensure consumer protection from the unknown yet potential effects of the presence of CEECs in water.

APPENDIX A: SOP FOR MONOCHLORAMINE DOSING SOLUTION PREPARATION

Standard Operating Procedure (SOP) for Monochloramine Dosing Solution Preparation and Residual Measurement

Prepared by Katja Kritsch edited by Yi Liu

Materials:

1. Laboratory grade water (LGW), purified using a secondary water purification system (Pure Water Solutions, Hillsborough, NC). Water pretreated with an general in-house purification system was pre-filtered (1 μm filter), treated to remove chlorine or chloramine residuals, passed through an activated carbon resin to reduce the total organic carbon content to less than 0.2 μg/L and passed through mixed-bed ion exchange resins to reduce the ion content to less than 18 MΩ.
2. Sodium hypochlorite (Fisher Scientific, Pittsburgh, PA, USA), 5 - 6%, concentration determined according to procedure **4500 Cl B. Iodometric Method I** in *Standard Methods for the Examination of Water and Wastewater, 2^{0th} edition*[1]
3. Ammonium sulfate (Fisher Scientific, Pittsburgh, PA, USA), granular, ≥99%, certified ACS
4. Sodium hydroxide (Fisher Scientific, Pittsburgh, PA, USA), 50% w/w/certified
5. Intermediate range pH test strips, EMD colorpHast (Fisher Scientific, Pittsburgh, PA, USA), pH 5 – 10

Glassware:

All glassware must be washed in detergent (Alconox), rinsed with tap water and LGW, soaked in a 10% Nitric acid solution over night, rinsed three times with LGW and dried in an 180 ℃ oven designated for glassware drying. Volumetric glassware must be rinsed three times with methanol and dried in the fume hood and cannot be place in the oven. Caps and Teflon cannot be acid washed. They are washed in a soap bath separate from glassware and each other, rinsed three times with tap water, three times with LGW and three times with methanol. To dry, caps and liners are placed on a clean Kimwipe, covered with another Kimwipe and dried in the fume hood.

- 125 mL amber Boston round bottles with open-top caps and Teflon-lined septa for (Laboratory Supply Distributors, Mt. Laurel, NJ, USA)
- 10 mL and 100 mL clear glass volumetric flasks with ground glass stoppers for stock solution preparation and UV measurement (Pyrex, Corning Inc., Corning, NY, USA)
- 250mL Clear Erlenmeyer flask (Pyrex, Corning Inc., Corning, NY, USA);

- 1 mL and 10 mL glass pipettes (Pyrex, Corning Inc., Corning, NY, USA)
- Pasteur pipettes
- Rubber bulbs
- 25mL glass burette (Pyrex, Corning Inc., Corning, NY, USA) for free chlorine titration
- Glass rod for stirring

Instruments and Additional Materials:

- Analytical Balance
- UV vis spectrometer
- Quartz cuvettes
- Kimwipe
- Lens wipes
- Burette Stand
- Stir plate and Teflon-coated stir bar for titration
- Hexagonal polystyrene weighing dishes

Solution Preparation:

NaOH Solution (1M) for pH adjustment

1. Fill a 25mL volumetric flask half full with LGW.
2. Add 1.3mL of 50% w/w NaOH into the volumetric flask.
3. Fill the volumetric flask to the mark with LGW, stopper and invert three times to mix.

24mM Ammonium Sulfate Solution (Stock Solution)

1. Tare one polystyrene weighing dish on the analytical balance and accurately weigh 0.128 g dry ammonium sulfate.
2. Quantitatively transfer solids into a clean, dry 100 mL volumetric flask.
3. Use a Pasteur pipette to carefully rinse any solids remaining on the weighing dish into the volumetric flask using LGW by slightly tipping the weighing dish slightly for the LGW to drain into the volumetric flask.
4. Carefully rinse any residuals on the funnel into the volumetric flask with LGW.
5. Swirl flask gently until all solids are dissolved.
6. Fill the volumetric flask to the mark with LGW, stopper and invert three times to mix.
7. Adjust to pH 8 with 1M NaOH (Typically ~5 drops). Test pH with pH paper.

Chloramine Dosing Solution Preparation and Analysis:

Chloramine Dosing Solution Preparation

1. Transfer ammonium sulfate solution to a 250mL Erlenmeyer flask in an ice bath.
2. After the solution has cooled down, place stir bar in flask and place on stir plate to mix solutions lowly.
3. Determine the volume of hypochlorite stock solution to add to the ammonium sulfate solution using the following equation:

$$\text{volume (mL)} = \frac{1400\text{mg}Cl_2/\text{L} \times 100\text{mL}}{[Cl_2](\text{mg}Cl_2/\text{L})}$$

   where volume is the amount of hypochlorite stock to add, 1400 mgCl_2/L is the target stock concentration of the monochloramine solution, 100mL is the volume of ammonium sulfate solution, and [Cl_2] is the measured hypochlorite stock solution in mg/L as Cl_2.
4. Measure out this amount and add to the glass burette.
5. Open the burette very slowly and add the hypochlorite stock drop-wise to the flask.
6. When all hypochlorite solution is added, transfer the solution to a 125mL amber bottle with cap and PTFE-lined septa. Measure the concentration of the monochloramine solution following the instruction on the next section. Store solution at 4^{o}C if not used immediately.

Determination of the Chloramine Concentration in the Dosing Solution

1. Turn on the UV-Vis instrument at least 30 minutes prior to use to warm up the lamps.
2. Prepare a 1:20 dilution of the dosing solution by transferring 0.5 mL of the dosing solution into a 10 mL volumetric flask using a glass pipette and fill to the mark with LGW.
3. Stopper the flask and invert three times to mix.
4. Zero the instrument with LGW.
5. Transfer an appropriate volume of diluted dosing solution into the cuvette and measure the absorbance at the wavelengths 245 and 295 to determine both the monochloramine and dichloramine concentrations by solving simultaneous Beer's Law equations, as described by Schreiber and Mitch (2005).

$$c_{di} = M_{Cl_2} \times 10 \times 1000 \times \left(\frac{\left(A_{295} - \left(\frac{A_{245}}{\varepsilon_{m,245}} \times \varepsilon_{m,295} \right) \right)}{\left(\frac{\varepsilon_{di,295} - (\varepsilon_{di,245} \times \varepsilon_{m,295})}{\varepsilon_{m,245}} \right)} \right)$$

c_{di} = concentration dichloramine [mg/L]

M_{Cl2}= molar mass of Cl_2 [g/mol]

A_{245}= total absorbance measured at a wavelength of 245 nm

A_{295}= total absorbance measured at a wavelength of 295 nm

$\varepsilon_{di,245}$ = 208 = extinction coefficient of dichloramine at λ = 245 nm

$\varepsilon_{di,295}$ = 267 = extinction coefficient of dichloramine at λ = 295 nm

$\varepsilon_{m,245}$ = 445 = extinction coefficient of monochloramine at λ = 245 nm

$\varepsilon_{m,295}$ = 14 = extinction coefficient of monochloramine at λ = 295 nm

$$c_m = M_{Cl_2} \times 10 \times 1000 \times \left(\frac{A_{245}}{\varepsilon_{m,245}} - c_{di} \times \frac{\varepsilon_{di,245}}{\varepsilon_{m,245}} \right)$$

c_m = concentration dichloramine [mg/L]

6. Use the calculated concentration c_m to for dosing calculations. Be sure that the results give a low (or sometimes negative due to the error range of the method) number for dichloramine if "pure" monochloramine dosing solution is desired.
7. Free chlorine and free ammonia may be determined using the Hach colorimeter and the appropriate procedures. However, it is not likely that free chlorine or free ammonia is present with a 3:1 chlorine-to-ammonia ratio. A dosing solution with a monochloramine concentration close to 1500 mg/L indicates that the preparation of pure monochloramine was successful. In case that a higher chlorine-to-ammonia ratio is employed it is recommended that the free chlorine concentration in the dosing solution is determined. It is also likely that a considerable concentration of dichloramine will be present.

References:

American Public Health Association, American Water Works Association, and Water Environment Federation, 1999. Standard Methods for the Examination of Water and Wastewater. 20th Edition, American Public Health Association: Washington DC.

Schreiber, I.M. & Mitch. W.A., 2005. Influence of the order of reagen addition on NDMA formation during chloramination. *Environmental Science & Technology*, 39(10). pp.3811-3818.

APPENDIX B: SUPPLEMENTARY DATA

Table B-1: LC-MS/MS ion count response for CEECs. RW = raw water, RWB = raw water spiked before filtering, RWA = raw water spiked after filtering.

Compound	1 mg/L standard	RW spiked 0.25mg/L	RW spiked 0.5mg/L	RW spiked 1mg/L	RWB	RWA
Caffeine	1.57E+06	2.30E+06	4.70E+06	7.72E+06	6.73E+06	1.19E+07
Fluoxetine	8.67E+07	2.65E+07	4.91E+07	8.82E+07	6.17E+07	8.44E+07
Atorvastatin	9.45E+05	4.12E+05	6.90E+05	1.20E+06	6.37E+05	5.53E+05
Iohexol	1.10E+06	3.81E+05	5.28E+05	1.11E+06	5.70E+05	6.61E+05
TBBPA	1.86E+06	7.59E+05	1.55E+06	2.41E+06	0.00E+00	2.62E+06

Table B-2: LC-MS/MS area response for CEECs. RW = raw water, RWB = raw water spiked before filtering, RWA = raw water spiked after filtering.

Compound	1 mg/L standard	RW spiked 0.25mg/L	RW spiked 0.5mg/L	RW spiked 1mg/L	RWB	RWA
Caffeine	3.47E+07	5.46E+07	8.60E+07	1.51E+08	1.21E+08	1.94E+08
Fluoxetine	1.22E+09	3.68E+08	6.48E+08	1.19E+09	7.70E+08	1.16E+09
Atorvastatin	1.21E+07	4.31E+06	7.40E+06	1.20E+07	6.73E+06	7.08E+06
Iohexol	1.21E+07	3.04E+06	6.69E+06	1.18E+07	5.65E+06	7.58E+06
TBBPA	2.04E+07	9.60E+06	1.83E+07	2.75E+07	0.00E+00	2.82E+07

Table B-3: LC-MS/MS relative area response for CEECs. RW = raw water

Compound	1 mg/L standard	RW spiked 0.25mg/L	RW spiked 0.5mg/L	RW spiked 1mg/L
Caffeine	10.1	36.3	74.8	145
Fluoxetine	35	244	563	1150
Atorvastatin	3.54	2.86	6.44	11.5
Iohexol	3.53	2.02	5.82	11.3

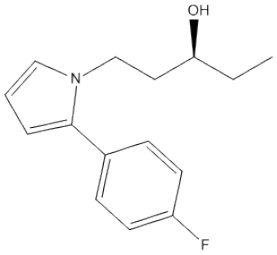
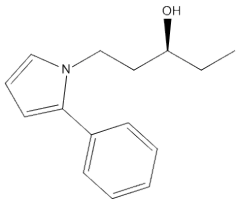
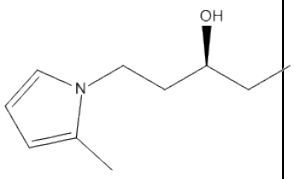
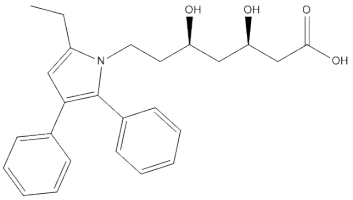
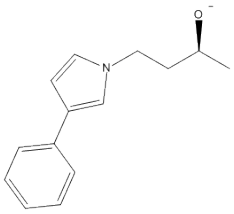
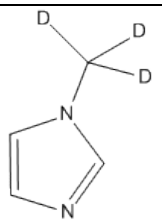
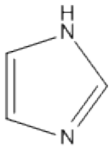
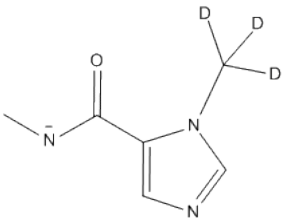
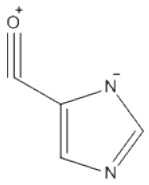
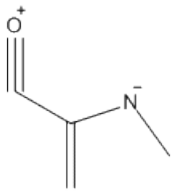
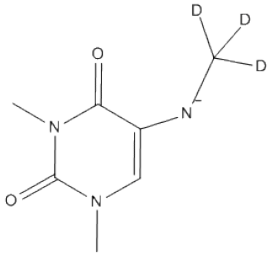
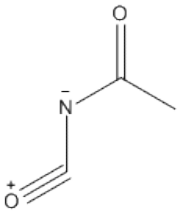
Table B- 4: Turbidity (NTU) of preliminary coagulation experiment

Dose (mg/L)	Aluminum Sulfate			Ferric Sulfate		
	Settling time			Settling time		
	10 min	30 min	60 min	10min	30 min	60 min
0	1.19	1.05	1.02	1.07	1.1	1
20	1.2	0.48	0.39	3.71	3.62	3.36
30	0.99	0.38	0.3	2.81	1.65	1.37
40	1.19	0.45	0.38	2.05	1.27	1.15
50	0.66	0.25	0.23	1.9	0.95	0.9
60	1.04	0.37	0.44	1.64	1.15	1.02

Table B-5: Comparison between quality control samples and samples for coagulation data (ng/L). Highlighted samples are outliers.

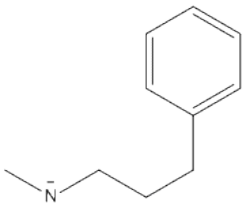
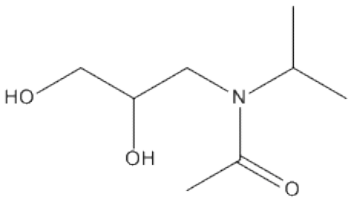
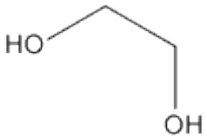
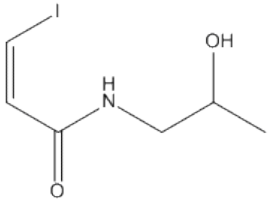
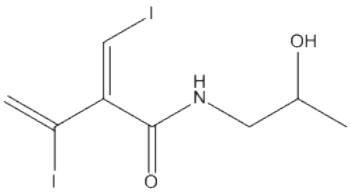
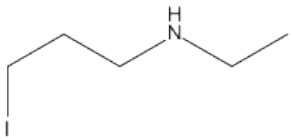
	Alum treated			Ferric treated			Before treatment		
	1	**2**	**QC-250**	**1**	**2**	**QC-250**	**1**	**2**	**QC-250**
Caffeine-d3	1149	684	934	1522	1170	928	912	1199	1155
Fluoxetine	515	528	498	416	454	266	554	333	841
Tetracycline	1066	584	547	1421	1279	1096	333	801	837
Atorvastatin	1579	1698	1161	1188	1267	751	1646	1132	1656
Iohexol	1558	1739	1500	1447	1508	1153	1945	1422	2634

Table B-6: Possible structures for daughter ions of chlorination byproducts.

Compound	Byproduct	Byproduct daughter ion 1	Byproduct daughter ion 2
Atorvastatin	 247.1	 229.0	 167.0
	 407.0	 214.0	N/A
Caffeine d3	 86.0	 68.7	N/A
	 141.0	 94.7	 83.1
	 171.0	152.3	 85.8

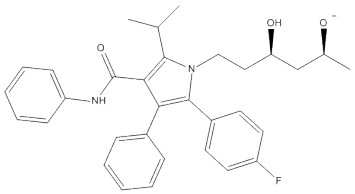
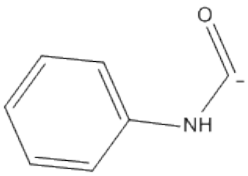
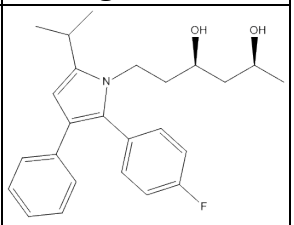
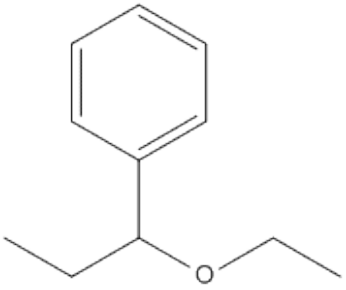
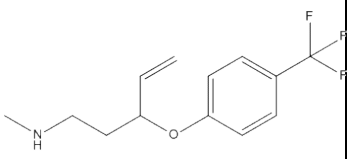
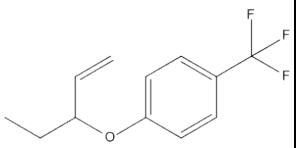
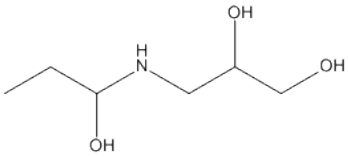
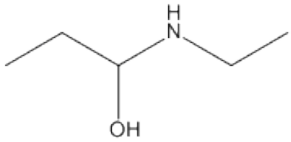
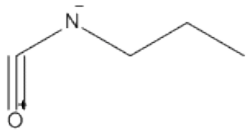
N/A= Not available

Table B-6 (continued): Possible structures for daughter ions of chlorination byproducts.

Compound	Byproduct	Byproduct daughter ion 1	Byproduct daughter ion 2
Fluoxetine	 148.2	102.9	69.0
Iohexol	 175.0	130.8	 62.8
	 255.1	156.9	144.8
	 407.0	 214.9	N/A

N/A= Not available

Table B-7: Possible structures for daughter ions of UV byproducts.

Compound	Byproduct	Byproduct daughter ion 1	Byproduct daughter ion 2
Atorvastatin	 513.2	 120.0	 395.1
Fluoxetine	 164.1	144.2	109.1
	 259.1	 230.8	90.8
Iohexol	 149.0	 103.1	 84.8

N/A: Not available

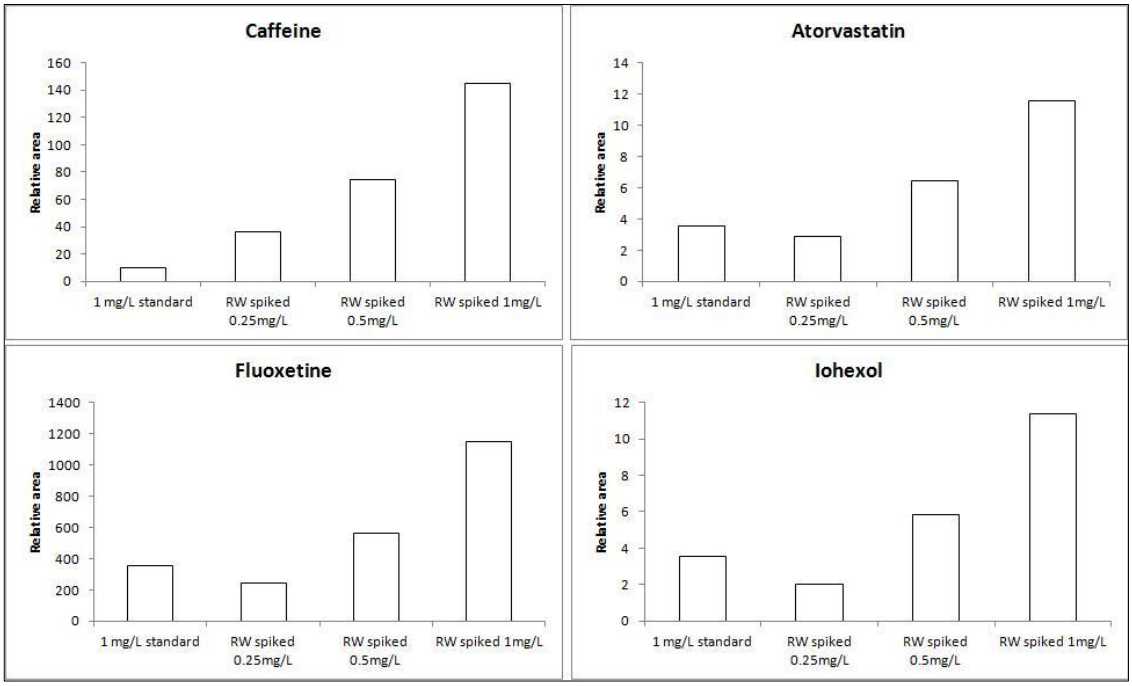


Figure B-1: Comparison of LC-MS/MS relative area response for SPE extraction of CEECs. RW = raw water.

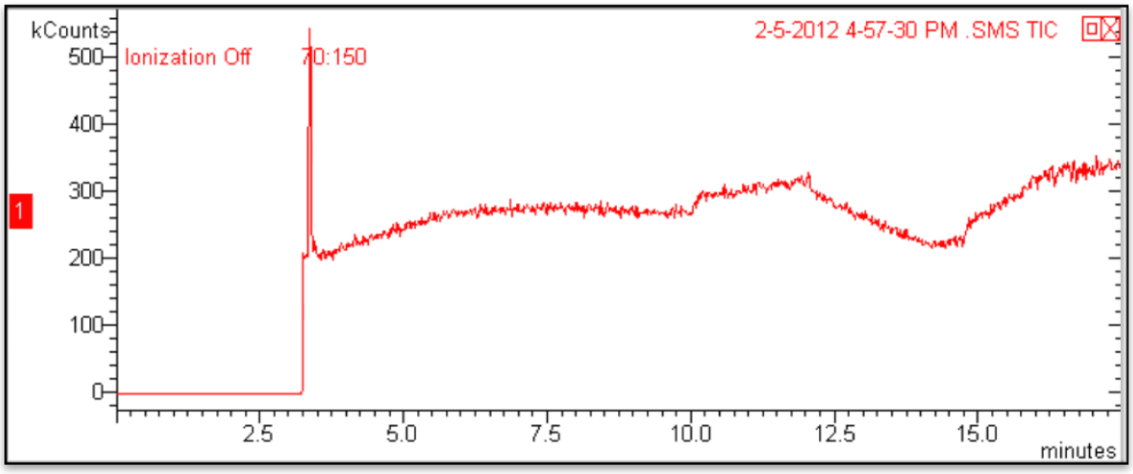


Figure B-2: Chromatographic peak of chlorinated tetracycline on GC-MS.

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